VERIFICATION OF TRANSLATION

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declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation made by me to the best of my knowledge and belief of:-

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[Document Name] Specification

[Title of the Invention] NOVEL HEMOPOIETIN RECEPTOR PROTEINS

[Claims]

[Claim 1] A protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.

[Claim 2] A protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.

[Claim 3] A protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.

[Claim 4] A protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7.

[Claim 5] A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 2, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.

[Claim 6] A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 4, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.

[Claim 7] A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 6, which is functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.

[Claim 8] A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 8, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7.

[Claim 9] A fusion protein comprising the protein of any one of claims 1 to 8 and another peptide or polypeptide.

[Claim 10] A DNA encoding the protein of any one of claims 1 to 9.

[Claim 11] A vector comprising the DNA of claim 10.

[Claim 12] A transformant harboring the DNA of claim 10 in an expressible manner.

[Claim 13] A method of producing the protein of any one of claims 1 to 8, comprising the step of culturing the transformant of claim 12.

[Claim 14] A method of screening a compound that binds to the protein of any one of claims 1 to 9 comprising the steps of:

(a) contacting a test sample with the protein of any one of claims 1 to 9; and

(b) selecting a compound that comprises an activity to bind to the protein of any one of claims 1 to 9.

[Claim 15] An antibody that specifically binds to the protein of any one of claims 1 to 8.

[Claim 16] A method of detecting or measuring the protein of any one of claims 1 to 9 comprising the steps of contacting a test sample presumed to contain said protein with the antibody of claim 15, and detecting or measuring the formation of the immune complex

between the antibody and the protein.

[Claim 17] A DNA specifically hybridizing to a DNA comprising a nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, and 8 comprising at least 15 nucleotides, and comprising at least 15 nucleotides.

[Detailed Description of the Invention]

[0001]

[Technical Field of Industrial Application]

The present invention relates to novel hemopoietin receptor proteins, the encoding genes, and methods of production and uses thereof.

[0002]

[Prior Art]

A large number of cytokines are known as humoral factors that are involved in the proliferation/differentiation of various cells, or activation of differentiated mature cells, and also cell death. These cytokines have their own specific receptors, which are categorized into several families based on their structural similarities (Hilton D.J., in "Guidebook to Cytokines and Their Receptors" edited by Nicola N.A. (A Sambrook & Tooze Publication at Oxford University Press), 1994, p8-16).

[0003]

Compared to similarities between receptors, primary-structure homology is quite low between cytokines, and a significant amino acid homology cannot be seen even among cytokine members that belong to the same receptor family. This explains the functional specificity of each cytokine, as well as similarities of cellular reactions induced by each cytokine.

[0004]

Representative examples of the above-mentioned receptor families are the tyrosine kinase receptor family, hemopoietin receptor family, tumor necrosis factor (TNF) receptor family, and transforming growth factor β (TGF β) receptor family. Different signal transduction pathways have been reported to be involved in each of these families. Among these receptor families, many receptors of especially the hemopoietin receptor family are expressed in blood cells and immunocytes, and their ligands, cytokines, are often termed as

hemopoietic factors or interleukins. Some of these hemopoietic factors or interleukins exist within blood and are thought to be involved in a systemic humoral regulation of hemopoietic or immune functions.

[0005]

This contrasts with the belief that cytokines belonging to other families are often involved in only topical regulations. Some of these hemopoietins can be taken as hormone-like factors, and conversely, representative peptide hormones such as the growth hormone, prolactin, or leptin receptors also belong to the hemopoietin receptor family. Because of these hormone-like systemic regulatory features, it is anticipated that hemopoietin administration would be applied in the treatment of various diseases.

[0006]

Among the large number of cytokines, those that are actually being clinically applied are, erythropoietin, G-CSF, GM-CSF, and IL-2. Combined with IL-11, LIF, and IL-12 that are being considered for clinical trials, and the above-mentioned peptide hormones such as growth hormone and prolactin, it can be envisaged that by searching among the above-mentioned various receptor families for a novel cytokine that binds to hemopoietin receptors, it is possible to find a cytokine that can be clinically applied with a higher efficiency.

[0007]

As mentioned above, cytokine receptors have structural similarities between the family members. Using these similarities, many investigations are being carried out aiming at finding novel receptors. Regarding the tyrosine kinase receptor especially, many receptors have already been cloned using its highly conserved sequence at the catalytic site (Matthews W. et al., Cell, 1991, 65 (7) p1143-52). Compared to this, hemopoietin receptors do not have a tyrosine kinase-like enzyme activity domain in their cytoplasmic regions, and their signal transductions are known to be mediated through associations with other tyrosine kinase proteins existing freely in the cytoplasm.

[0008]

Though the binding site on receptors associating with these cytoplasmic tyrosine kinases (JAK kinases) is conserved between family

members, the homology is not very high (Murakami M. et al., Proc. Natl. Acad. Sci. USA, 1991, 88, 11349-11353). On one hand, the sequence that characterizes these hemopoietin receptors most well exists in the extracellular region, and especially the five amino acid Trp-Ser-Xaa-Trp-Ser (where Xaa is an arbitrary amino acid) motif is conserved in almost all of the hemopoietin receptors. Therefore, novel receptors are expected to be obtained by searching novel family members using this sequence. In fact, this approach has already identified the IL-11 receptor (Robb, L. et al., J. Biol. Chem., 1996, 271 (23) 13754-13761), leptin receptor (Gainsford T. et al., Proc. Natl. Acad. Sci. USA, 1996, 93 (25) p14564-8) and the IL-13 receptor (Hilton D.J. et al., Proc. Natl. Acad. Sci. USA, 1996, 93 (1) p497-501).

[0009]

[Problems to Be Solved by the Invention]

The present invention provides a novel hemopoietin receptor protein, and the encoding DNA. The present invention also provides, a vector into which the DNA has been inserted, a transformant harboring the DNA, and a method of producing a recombinant protein using the transformant. It also provides a method of screening a compound that binds to the protein.

[0010]

[Means to Solve the Problems]

Until now, the inventors have been trying to search for a novel receptor using an oligonucleotide encoding the Trp-Ser-Xaa-Trp-Ser motif as a probe by plaque hybridization, RT-PCR method, and so on. However, because of reasons such as the oligonucleotide tggag (t/c) nnntggag (t/c) (where n is an arbitrary nucleotide) that encodes the motif being short having just 15 nucleotides, and the g/c being high, it was extremely difficult to strictly select only those in which the 15 nucleotides have completely hybridized under the usual hybridization conditions.

[0011]

Also, a similar sequence is contained within cDNA encoding proteins other than hemopoietin receptors, starting with various collagens that are thought to be widely distributed and also have high expression amounts, which makes the screening by the above-mentioned plaque

hybridization and RT-PCR highly inefficient.

[0012]

To solve these problems, and to estimate how many different hemopoietic receptor genes actually exist on the human genome, the inventors computer-searched sequences that completely coincided with each probe using all capable oligonucleotide sequences encoding the above-mentioned Trp-Ser-Xaa-Trp-Ser motif as probes.

[0013]

Next, among the clones identified by the above search, the nucleotide sequence around the probe sequence of human genome-derived clones (cosmid, BAC, PAC) was converted to the amino acid sequence and compared with the amino acid sequence of known hemopoietin receptors to select genes thought to encode hemopoietin receptor family members.

[0014]

From the above search, two clones thought to be hemopoietin receptor genes were identified. One of these was the known GM-CSFβ receptor gene (derived from the 22q12.3-13.2 region of chromosome no. 22), and the other (BAC clone AC002303 derived from the 16p12 region of chromosome no. 16) was presumed to encode a novel hemopoietin receptor protein, and this gene was named "NR8."

[0015]

Next, the cDNA thought to encode NR8 was found within the human fetal liver cell cDNA library by RT-PCR using a specific primer designed based on the obtained nucleotide sequence. Furthermore, using this cDNA library as the template, the full-length cDNA NR8α encoding a transmembrane receptor comprising 361 amino acids was ultimately obtained by 5'-RACE method and 3'-RACE method.

[0016]

In the primary structure of NR8α, a cysteine residue and a proline rich motif conserved between other family members, were well conserved in the extracellular region, and in the intracellular region, the Box 1 motif thought to be involved in signal transduction was well conserved, and therefore, NR8α was thought to be a typical hemopoietin receptor.

[0017]

Furthermore, the inventors revealed the presence of two genes named

NR8β and NR8γ as selective splicing products of NR8α.

[0018]

Therefore, the present invention provides (1) a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.

[0019]

The present invention also provides (2) a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.

[0020]

The present invention also provides (3) a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.

[0021]

The present invention also provides (4) a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser

of SEQ ID NO: 7.

[0022]

The present invention also provides (5) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 2, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.

[0023]

The present invention also provides (6) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 4, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.

[0024]

The present invention also provides (7) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 6, which is functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.

[0025]

The present invention also provides (8) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 8, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7.

[0026]

The present invention also provides (9) a fusion protein comprising the protein of any one of (1) to (8) and another peptide or polypeptide.

[0027]

The present invention also provides (10) a DNA encoding the protein of any one of (1) to (9).

[0028]

The present invention also provides (11) a vector comprising the DNA of (10).

[0029]

The present invention also provides (12) a transformant harboring

the DNA of (10) in an expressible manner.

[0030]

The present invention also provides (13) a method of producing the protein of any one of (1) to (8), comprising the step of culturing the transformant of (12).

[0031]

The present invention also provides (14) a method of screening a compound that binds to the protein of any one of (1) to (9) comprising the steps of:

(a) contacting a test sample with the protein of any one of (1) to (9); and

(b) selecting a compound that comprises an activity to bind to the protein of any one of (1) to (9).

[0032]

The present invention also provides (15) an antibody that specifically binds to the protein of any one of (1) to (8).

[0033]

The present invention also provides (16) a method of detecting or measuring the protein of any one of (1) to (9) comprising the steps of contacting a test sample presumed to contain said protein with the antibody of (15), and detecting or measuring the formation of the immune complex between the antibody and the protein.

[0034]

The present invention also provides (17) a DNA specifically hybridizing to a DNA comprising a nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, and 8 comprising at least 15 nucleotides, and comprising at least 15 nucleotides.

[0035]

[Mode for Carrying Out the Invention]

The present invention relates to the novel hemopoietin receptor "NR8." 5'-RACE and 3'-RACE analyses, NR8 genome sequence analysis, and plaque screening analysis revealed the presence of NR8α, NR8β, and NR8γ. The structures of these NR8 genes are shown in Fig. 13. Among the NR8 genes, NR8β is an alternative splicing product lacking the 5^{th} exon, and can encode two different proteins, a soluble protein in which the CDS ends with a stop codon on the 6^{th} exon that results

from a frame shift following direct coupling to the 4th exon, and a membrane-bound protein lacking the signal sequence starting from the ATG upon the 4th exon.

[0036]

Since the soluble protein comprises the same sequence as NR8α up to the 4th exon, it may function as a soluble receptor. On the other hand, NR8γ encodes a protein containing a 177 amino acid insertion derived from the NR8 9th intron close to the C terminus of the NR8α as a result of selective splicing.

[0037]

Both NR8α and NR8γ encode transmembrane-type hemopoietin receptors. Among the sequences conserved between other hemopoietin receptors that are thought to be involved in signal transduction, a motif resembling Box 1 exists in the intracellular domain of NR8α and NR8γ adjacent to the cell membrane. Though low in the degree of conservation, a sequence that is similar to Box 2 also exists, and therefore, NR8 is thought to be a type of receptor in which the signal is transduced by a homodimer.

[0038]

The amino acid sequences of the NR8 proteins included in the proteins of the present invention are shown in SEQ ID NO: 1 (NR8α), SEQ ID NO: 3 (soluble NR8β), SEQ ID NO: 5 (membrane-bound NR8β), and SEQ ID NO: 7 (NR8γ), and the nucleotide sequences of cDNA encoding these proteins are shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8, respectively.

[0039]

Northern blotting for the spleen, thymus, peripheral leucocytes, and lung showed two to three bands in the 5kb and 3 to 4kb regions. Similar sized bands were observed for cell lines HL60 and Raji also, but no expression was seen for other tumor cell lines (HeLa, SW480, A549, G361) and leukemia cell lines (K562, MOLT4).

[0040]

The above results suggest that NR8 is specifically expressed on hemopoietic cell lines, especially on granulocytic lines, and B cell lines.

[0041]

The above NR8 protein is expected to be applied in medicine. NR8 is expressed in fetal liver, spleen, thymus, and some leukemic cell lines, suggesting the possibility that it might be a receptor of an unknown hemopoietic factor. Therefore, NR8 protein would be a useful material for obtaining this unknown hemopoietic factor.

[0042]

Furthermore, it is possible that NR8 is specifically expressed in limited cell populations within these hemopoietic tissues, and therefore, anti NR8 antibody may be useful as a means of separating these cell populations. Thus separated cell populations can be applied for cell transplant therapy. Anti NR8 antibody is also expected to be applied for the diagnosis and treatment of leukemic diseases represented by leukemia.

[0043]

On the other hand, the soluble protein including the extracellular domain of NR8 protein, or NR8β, a splicing variant of NR8, may be applied as a decoy-type receptor that is an inhibitor of the NR8 ligand, and is anticipated to be applied in the treatment of diseases in which NR8 is involved, starting with leukemia.

[0044]

The present invention also encompasses a protein that is functionally equivalent to the above-mentioned NR8 protein. Herein "functionally equivalent" means having an equivalent biological activity to the above-mentioned NR8 proteins. Hemopoietic factor receptor protein activity can be given as an example of a biological activity. Such proteins can be obtained by the method of introducing a mutation to the amino acid sequence of a protein. For example, site-specific mutagenesis using a synthetic oligonucleotide primer, can be used to introduce a desired mutation into the amino acid sequence of a protein (Kramer, W. and Fritz, H.J., Methods in Enzymol., 1987, 154, 350-367). This could also be done by a PCR-mediated site-specific mutagenesis system (GIBCO-BRL). Using these methods, the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7 can be modified to obtain a protein functionally equivalent to the NR8 protein, in which one or more amino acids in the amino acid sequence of the protein have been deleted, added, and/or substituted by another

amino acid without affecting the biological activity of the protein.

[0045]

As a protein functionally equivalent to the NR8 protein of the invention, the following are given: one in which one or two or more, preferably, two to 30, more preferably, two to ten amino acids are deleted in any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5; one in which one or two or more, preferably, two to 30, more preferably, two to ten amino acids have been added into any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; or one in which one or two or more, preferably, two to 30, more preferably, two to ten amino acids have been substituted with other amino acids in any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7.

[0046]

It is already known that a protein comprising a modified amino acid sequence of a certain amino acid sequence in which one or more amino acid residues have been deleted, added, and/or substituted with another amino acid, still maintains its biological activity (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA, 1984, 81, 5662-5666; Zoller, M. J. & Smith, M., Nucleic Acids Research, 1982, 10, 6487-6500; Wang, A. et al., Science, 224, 1431-1433; Dalbadie-McFarland, G. et al., Proc. Natl. Acad. Sci. USA, 1982, 79, 6409-6413).

[0047]

For example, a fusion protein can be given as a protein in which one or more amino acid residues have been added to the NR8 protein of the present invention. A fusion protein is made by fusing the NR8 protein of the present invention with another peptide or protein and is encompassed in the present invention. A fusion protein can be prepared by ligating DNA encoding the NR8 protein of the present invention with DNA encoding another peptide or protein so as the frames match, introducing this into an expression vector, and expressing the fusion gene in a host. Methods commonly known can be used for preparing such a fusion gene. There is no restriction as to the other peptide or protein that is fused to the protein of this invention.

[0048]

For example, FLAG (Hopp, T.P. et al., Biotechnology, 1988, 6, 1204-1210), 6x His constituting six histidine (His) residues, 10x His, Influenza agglutinin (HA), human c-myc fragment, VSV-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, lck tag, α-tubulin fragment, B-tag, Protein C fragment, and such well-known peptides can be used. Examples of proteins are, glutathione-S-transferase (GST), Influenza agglutinin (HA), immunoglobulin constant region, β-galactosidase, maltose-binding protein (MBP), etc. Commercially available DNAs encoding these may also be used to prepare fusion proteins.

[0049]

The protein of the invention can also be encoded by a DNA that hybridizes under stringent conditions to a DNA comprising any one of the nucleotide sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8. Such a protein also includes a protein functionally equivalent to the above-mentioned NR8 protein. Stringent conditions can be suitably selected by one skilled in the art, and for example, low-stringent conditions can be given. Low-stringent conditions are, for example, 42℃, 2x SSC, and 0.1% SDS, and preferably, 50℃, 2x SSC, and 0.1% SDS. More preferable are highly stringent conditions, for example, 65℃, 2x SSC, and 0.1% SDS. Under these conditions, the higher the temperature is raised, the higher the homology of the obtained DNA will be.

[0050]

The present invention also includes a protein that is functionally equivalent to the above NR8 protein, which has also a homology with a protein comprising any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7. A protein having a homology means, a protein having at least 70%, preferably at least 80%, more preferably at least 90%, even more preferably, at least 95% homology to any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7. The homology of a protein can be determined by the algorithm in "Wilbur, W.J. and Lipman, D.J. Proc. Natl. Acad. Sci. USA, 1983, 80, 726-730."

[0051]

In the protein of the invention, the amino acid sequence, molecular

weight, isoelectric point, the presence or absence of the sugar chain, and its form differ according to the producing cells, host, or purification method described below. However, as long as the obtained protein comprises a hemopoietic factor receptor protein activity, it is included in the present invention.

[0052]

For example, if the protein of the present invention is expressed in prokaryotic cells such as *E. coli*, a methionine residue is added at the N-terminus of the amino acid sequence of the expressed protein. If the protein of the present invention is expressed in eukaryotic cells such as mammalian cells, the N-terminal signal sequence is removed. The protein of the present invention includes these proteins.

[0053]

For example, as a result of analyzing the protein of the invention based on the method in "Von Heijne, G., Nucleic Acids Research, 1986, 14, 4683-4690," it was presumed that the signal sequence is from the 1st Met to the 19th Gly in the amino acid sequence of SEQ ID NO: 1. Therefore, the present invention encompasses a protein comprising the sequence from the 20th Cys to 361st Ser in the amino acid sequence of SEQ ID NO: 1.

[0054]

To produce the protein of the invention, the obtained DNA is incorporated into an expression vector in a manner that the DNA is expressible under the regulation of an expression regulatory region, for example, an enhancer or promoter. Next, host cells are transformed by this expression vector to express the protein.

[0055]

Specifically, the protein can be produced as follows. When mammalian cells are used, DNA comprising a commonly used useful promoter/enhancer, DNA encoding the protein of the invention, and the poly A signal that is functionally bound to the 3′ side downstream of the protein-encoding DNA, or a vector containing it, is constructed. For example, as the promoter/enhancer, human cytomegalovirus immediate early promoter/enhancer can be given.

[0056]

Also, as other promoters/enhancers that can be used for protein

expression, viral promoters/enhancers of retroviruses, polyomaviruses, adenoviruses, simian virus 40 (SV40), and such, and promoters/enhancers derived from mammalian cells, such as that of human elongation factor 1α (HEF1α) can be used.

[0057]

For example, a protein can be easily expressed by following the method of Mulligan et al. (Nature, 1979, 277, 108) when using the SV40 promoter/enhancer, and the method of Mizushima et al. (Nucleic Acids Res., 1990, 18, 5322) when using the HEF1α promoter/enhancer.

[0058]

When using *E. coli*, well-used useful promotors, the signal sequence for polypeptide secretion, and genes to be expressed, may be functionally bound to express the desired gene. For example, lacZ promoter and araB promoter may be used as promotors. When using the lacZ promoter, the method of Ward et al. (Nature, 1098, 341, 544-546; FASEB J., 1992, 6, 2422-2427), and when using the araB promoter, the method of Better et al. (Science, 1988, 240, 1041-1043) may be followed.

[0059]

When producing the protein into the periplasm of *E. coli*, the pelB (Lei, S. P. et al., J. Bacteriol., 1987, 169, 4379) signal sequence may be used as a protein secretion signal.

[0060]

A replication origin derived from SV40, polyomavirus, adenovirus, bovine papilomavirus (BPV), and such may be used. To amplify gene copies in host cell lines, the expression vector may include an aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, *E.coli* xanthine guanine phosphoribosyl transferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, and such as a selective marker.

[0061]

The expression vector used to produce the protein of the invention may be any, as long as it's an expression vector that is suitably used for the present invention. Mammalian expression vectors, for example, pEF and pCDM8; insect-derived expression vectors, for example, pBacPAK8; plant-derived expression vectors, for example, pMH1 and pMH2; animal virus-derived expression vectors, for example, pHSV, pMV, and pAdexLcw; retrovirus-derived expression vectors, for example,

pZIpneo; yeast-derived expression vectors, for example, pNV11 and SP-Q01; *Bacillus subtilis*-derived expression vectors, for example, pPL608 and pKTH50; *E. coli*-derived expression vectors, for example, pQE, pGEAPP, pGEMEAPP, and pMALp2 can be given as expression vectors of this invention.

[0062]

Not only vectors of the present invention that produce the protein of the invention *in vivo* and *in vitro*, but also those that are used for gene therapy of mammals, for example humans.

[0063]

When introducing the expression vector of the present invention constructed above into a host cell, well-known methods, for example the calcium phosphate method (Virology, 1973, 52, 456-467), electroporation (EMBO J., 1982, 1, 841-845), and such may be used.

[0064]

In the present invention, an arbitrary production system may be used to produce the protein. *In vitro* and *in vivo* production systems are known as production systems for producing proteins. Production systems using eukaryotic cells and prokaryotic cells may be used as *in vitro* production systems.

[0065]

When using eukaryotic cells, production systems using, for example, animal cells, plant cells, and fungal cells are known. As animal cells used, for example, mammalian cells such as CHO (J. Exp. Med., 1995, 108, 945), COS, myeloma, baby hamster kidney (BHK), HeLa, or Vero, amphibian cells such as *Xenopus* oocytes (Valle, et al., Nature, 1981, 291, 358-340), insect cells such as sf9, sf21, or Tn5, are known. As CHO cells, especially DHFR gene-deficient CHO cell, dhfr-CHO (Proc. Natl. Acad. Sci. USA, 1980, 77, 4216-4220), and CHO K-1 (Proc. Natl. Acad. Sci. USA, 1968, 60, 1275) can be suitably used.

[0066]

Nicotiana tabacum-derived cells are well known as plant cells, and these can be callus cultured. As fungal cells, yeasts such as the *Saccharomyces* genus, for example, *Saccharomyces cerevisiae*, filamentous bacteria such as the *Aspergillus* genus, for example, *Aspergillus niger* are known.

[0067]

Bacterial cells may be used as prokaryotic production systems. As bacterial cells, *E. coli* and *Bacillus subtilis* are known.

[0068]

Proteins can be obtained by transforming these cells with the objective DNA, and culturing the transformed cells *in vitro* according to well-known methods. For example, DMEM, MEM, RPMI1640, and IMDM can be used as culture media. At that instance, fetal calf serum (FCS) and such serum supplements may be added in the above media, or a serum-free culture medium may be used. The pH is preferably about 6 to 8. Culture is usually done at about 30℃ to 40℃, for about 15 to 200 hr, and medium changes, aeration, and stirring are done as necessary.

[0069]

On the other hand, production systems using animals and plants may be given as *in vivo* production systems. The objective gene is introduced into the plant or animal, and the protein is produced within the plant or animal, and recovered. "Host" as used in the present invention encompasses such animals and plants as well.

[0070]

When using animals, mammalian and insect production systems can be used. As mammals, goats, pigs, sheep, mice, and cattle may be used (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). Transgenic animals may also be used when using mammals.

[0071]

For example, the objective DNA is inserted within a gene encoding a protein produced intrinsically into milk, such as goat β casein, to prepare a fusion gene. The DNA fragment containing the fusion gene is injected into a goat's embryo, and this embryo is implanted in a female goat. The protein is collected from the milk of the transgenic goats produced from the goat that received the embryo, and descendents thereof. To increase the amount of protein-containing milk produced from the transgenic goat, a suitable hormone/hormones may be given to the transgenic goats (Ebert, K.M. et al., Bio/Technology, 1994, 12, 699-702).

[0072]

Silk worms may be used as insects. When using the silk worm, it is infected with a baculovirus to which the objective DNA has been inserted, and the desired protein is obtained from the body fluids of the silk worm (Susumu, M. et al., Nature, 1985, 315, 592-594).

[0073]

When using plants, for example, tobacco can be used. In the case of tobacco, the objective DNA is inserted into a plant expression vector, for example pMON 530, and this vector is introduced into a bacterium such as *Agrobacterium tumefaciens*. This bacterium is infected to tobacco, for example *Nicotiana tabacum*, to obtain the desired polypeptide from tobacco leaves (Julian, K.-C. Ma et al., Eur. J. Immunol., 1994, 24, 131-138).

[0074]

The thus-obtained protein of the invention is isolated from within and without cells, or from hosts, and can be purified as a substantially pure homogenous protein. The separation and purification of the protein is not limited to any specific method and can be done using ordinary separation and purification methods used to purify proteins. For example, chromatography, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, recrystalization, and such may be suitably selected, or combined to separate/purify the protein.

[0075]

As chromatographies, for example, affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration, reversed-phase chromatography, adsorption chromatography, and such can be exemplified (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be done by liquid chromatography such as HPLC, FPLC, and the like. The present invention encompasses proteins highly purified by using such purification methods.

[0076]

Proteins can be arbitrarily modified, or peptides may be partially excised by treating the proteins with appropriate modification enzymes

prior to or after the purification. Trypsin, chymotrypsin, lysyl endopeptidase, protein kinase, glucosidase, and such are used as protein modification enzymes.

[0077]

The present invention includes a partial peptide comprising the active center of a protein comprising any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7. A partial peptide of the protein of the present invention is, for example, a partial peptide of the molecules of the protein, which contains one or more regions of the hydrophilic region and hydrophobic region presumed by hydrophobicity plot analysis. These partial peptides may contain the whole hydrophilic region or a part of it, and may contain the whole hydrophobic region or a part of it. For example, soluble proteins and proteins comprising extracellular regions of the protein of the invention, are also encompassed in the invention.

[0078]

The partial peptides of the protein of the invention may be produced by genetic engineering techniques, well-known peptide synthesizing methods, or by excising the protein of the invention by a suitable peptidase. As peptide synthesizing methods, the solid-phase synthesizing method, and the liquid-phase synthesizing method may be used.

[0079]

The present invention also relates to a DNA encoding the protein of the invention. A cDNA encoding the protein of the invention may be obtained by, for example, screening a human cDNA library using the probe described herein.

[0080]

Using the obtained cDNA or cDNA fragment as a probe, cDNA can also be obtained from other cells, tissues, organs, or species by further screening cDNA libraries. cDNA libraries may be prepared by, for example, the method of Sambrook, J. et al., Molecular Cloning, Cold Spring Harbor Laboratory Press (1989), or commercially available cDNA libraries may be used.

[0081]

By determining the nucleotide sequence of the obtained cDNA, the

translation region encoded by it can be determined, and the amino acid sequence of the protein of the present invention can be obtained. Furthermore, genomic DNA can be isolated by screening the genomic DNA library using the obtained cDNA as a probe.

[0082]

Specifically, this can be done as follows. First, mRNA is isolated from cells, tissues, and organs expressing the protein of the invention. For this mRNA isolation, whole RNA is prepared using well-known methods, for example, guanidine ultracentrifugation method (Chirgwin, J.M. et al., Biochemistry, 1979, 18, 5294-5299), the AGPC method (Chomczynski, P. and Sacchi, N., Anal. Biochem., 1987, 162, 156-159), and such, and purified using the mRNA Purification Kit (Pharmacia), etc. mRNA may be directly prepared using the QuickPrep mRNA Purification Kit (Pharmacia).

[0083]

cDNA is synthesized using reverse transcriptase from the obtained mRNA. cDNA can be synthesized using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (SEIKAGAKU CORPORATION), etc. Also, cDNA synthesis and amplification may also be done using the probe described herein by following the 5'-RACE method (Frohman, M.A. et al., Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 8998-9002; Belyavsky, A. et al., Nucleic Acids Res., 1989, 17, 2919-2932) using the polymerase chain reaction (PCR) and the 5'-Ampli FINDER RACE KIT (Clontech).

[0084]

The objective DNA fragment is prepared from the obtained PCR product and ligated with vector DNA. Thus, a recombination vector is created, introduced into *E.coli*, etc. and colonies are selected to prepare the desired recombination vector. The nucleotide sequence of the objective DNA may be verified by known methods, for example, the dideoxy nucleotide chain termination method.

[0085]

In the DNA of the invention, a sequence with a higher expression efficiency can be designed by considering the codon usage frequency of hosts used for the expression (Grantham, R. et al., Nucleic Acids Research, 1981, 9, p43-p74). The DNA of the invention may also be modified using commercially available kits and known methods. For

example, digestion by restriction enzymes, insertion of synthetic oligonucleotides and suitable DNA fragments, addition of linkers, insertion of a start codon (ATG) and/or stop codon (ATT, TGA, or TAG), and such can be given.

[0086]

The DNA of the present invention encompasses DNA comprising the nucleotide sequence from the 441st nucleotide A to the 1523rd nucleotide C in the nucleotide sequence of SEQ ID NO: 2, DNA comprising the nucleotide sequence from the 441st nucleotide A to the 872nd nucleotide A in the nucleotide sequence of SEQ ID NO: 4, DNA comprising the nucleotide sequence from the 659th nucleotide A to the 1368th nucleotide C in the nucleotide sequence of SEQ ID NO: 6, and DNA comprising the nucleotide sequence from the 441st nucleotide A to the 2054th nucleotide C in the nucleotide sequence of SEQ ID NO: 8.

[0087]

The DNA of the present invention encompasses DNA that hybridizes under stringent conditions to the DNA comprising any one of the nucleotide sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8, which also includes a DNA encoding a protein functionally equivalent to the NR8 protein.

[0088]

Stringent conditions can be suitably selected by one skilled in the art, and for example, low-stringent conditions can be given. Low-stringent conditions are, for example, 42℃, 2x SSC, and 0.1% SDS, and preferably 50℃, 2x SSC, and 0.1% SDS. More preferable are highly stringent conditions, for example, 65℃, 2x SSC, and 0.1% SDS. Under these conditions, the higher the temperature is raised, the higher the homology of the obtained DNA will be. The above DNA is preferably natural DNA such as cDNA and chromosomal DNA.

[0089]

As shown in Examples, the mRNA of the gene hybridizing to cDNA encoding the protein of the invention was distributed in various human tissues. Therefore, the above-mentioned natural DNA may be, for example, genomic DNA and cDNA derived from tissues in which the mRNA that hybridizes to the cDNA encoding the protein of the invention is detected in Examples. The DNA encoding the protein of the invention

may be cDNA, genomic DNA, or synthetic DNA.

[0090]

The protein of the invention is useful in screening a compound that binds to it. Namely, the protein of the invention is used in the screening method that comprises the steps of contacting a test sample expected to contain a compound that binds to the protein of the invention with the protein of the invention, and selecting the compound that comprises an activity to bind to the protein of the invention.

[0091]

As methods for screening a compound that comprises an activity to bind to the protein of the invention, numerous methods usually used by those skilled in the art can be employed. The protein of the invention that is used for the screening of the invention may be a recombinant, natural, or partial peptide. A compound comprising an activity to bind to the protein of the invention may be a protein comprising a binding activity, or it may be a chemically synthesized compound having a binding activity.

[0092]

As a test sample that is used in the screening method of the present invention, for example, peptides, purified or crudely purified proteins, non-peptide compounds, synthetic compounds, microbial fermentation products, extracts of marine organisms, plant extracts, cell extracts, animal tissue extracts, and such can be given. These test samples may be novel compounds, or well-known compounds.

[0093]

A protein that binds to the protein of the invention can be screened by, for example, using the West-western blotting method (Skolnik, E.Y. et al., Cell, 1991, 65, 83-90). cDNA is isolated from cells, tissues, and organs presumed to express the protein binding to the protein of the invention, this is inserted into phage vectors, for example, λgt11, ZAPII, and such, to make a cDNA library, expressed on a plate containing a culture medium, the proteins expressed are fixed on a filter, this filter is reacted with the labeled, purified protein of the invention, and plaques expressing the protein bound to the protein of the invention are detected by the labels. As methods

to label the protein of the invention, the method that uses the binding ability of avidin and biotin, the method of using an antibody that specifically binds to the protein of the invention or the peptide or polypeptide fused to the protein of the invention, the method of using radioisotopes, or fluorescence, and such can be given.

[0094]

A ligand that binds specifically to the protein of the invention can be screened by, preparing a chimeric receptor by ligating the extracellular domain of the protein of the invention with the intracellular domain containing the transmembrane domain of a hemopoietin receptor protein comprising a known signal transduction ability, expressing this chimeric receptor on the cell surface of a suitable cell line, preferably, a cell line that can survive and proliferate under the presence of a suitable growth factor (a growth factor-dependent cell line), and culturing the cell line by adding a material that is expected to contain various growth factors, cytokines, or hemopoietic factors. This method uses the fact that the above-mentioned growth factor-dependent cell line survives and proliferates only when a ligand that specifically binds to the extracellular domain of the protein of the invention exists within the test material. Known hemopoietic receptors are, for example, the thrombopoietin receptor, erythropoietin receptor, G-CSF receptor, gp130, etc. However, the partner of the chimeric receptor used in the screening of the invention is not limited to these known hemopoietic receptors, and any may be used as long as a structure needed for the signal transduction activity is contained in the cytoplasmic domain. Growth factor-dependent cell lines are for example, IL-3-dependent cell lines starting with BaF3 and FDC-P1.

[0095]

As a ligand that specifically binds to the protein of the invention, the possibility of not only soluble proteins, but also cell membrane-binding proteins can be envisaged, though rare. In such cases, screening can be done by labeling the protein containing only the extracellular domain of the protein of the invention, or a fusion protein in which the partial sequence of another soluble protein has been added to this extracellular domain, and measuring the binding

with cells expected to express the ligand. As examples of proteins containing only the extracellular domain of the protein of the invention, for example, a soluble receptor protein artificially made by inserting a stop codon to the N terminal side of the transmembrane domain, or NR8β soluble protein may be used. On the other hand, as a fusion protein in which the partial sequence of another soluble protein has been added to the extracellular domain of the protein of the invention, for example, proteins prepared by adding immunoglobulin Fc site, FLAG peptide, etc. to the C terminus of the extracellular domain can be used. These soluble labeled proteins can be used in the detection in the above-described West-western blotting method.

[0096]

A protein that binds to the protein of the invention can be screened by using the two-hybrid system (Fields, S. and Sternglanz, R., Trends. Genet., 1994, 10, 286-292).

[0097]

In the two-hybrid system, an expression vector containing DNA encoding the fusion protein between the protein of the invention and one subunit of a heterodimeric transcriptional regulatory factor, and an expression vector containing DNA made by ligating DNA encoding the other subunit of the heterodimeric transcriptional regulatory factor and a desired cDNA used as a test sample are introduced into cells and expressed. If the protein encoded by the cDNA binds with the protein of the invention and the transcriptional regulatory factor forms a heterodimer, a reporter gene constructed in the cell beforehand will be expressed. Therefore, a protein binding to the protein of the invention can be selected by detecting or measuring the expression level of the reporter gene.

[0098]

Specifically, the DNA encoding the protein of the invention and the gene encoding the DNA binding domain of LexA are ligated so as the frames match to prepare an expression vector. Next, the desired cDNA and the gene encoding GAL4 transcription activation domain are ligated to prepare an expression vector.

[0099]

Cells into which the HIS3 gene has been incorporated (the

transcription of HIS3 gene is regulated by the promoter having a LexA binding motif) are transformed by the above two-hybrid system expression plasmids, and then incubated on a histidine-free synthetic culture medium. Herein, cells only grow when a protein interaction is present. Thus, the increase in reporter gene expression can be examined by the growth rate of the transformant.

[0100]

Other than the HIS3 gene, for example, the luciferase gene, plasminogen activator inhibitor type1 (PAI-1) gene and such can be used as reporter genes.

[0101]

The two-hybrid system may be constructed according to the usual methods, or a commerciálly available kit may be used. As commercially available two-hybrid system kits, the MATCHMARKER Two-Hybrid System, Mammalian MATCHMARKER Two-Hybrid Assay Kit (both by CLONTEC), and HybriZAP Two-Hybrid Vector System (Stratagene) can be given.

[0102]

A protein binding to the protein of the invention can be screened by affinity chromatography. Namely, the protein of the invention is immobilized onto a carrier of an affinity column, and a test sample presumed to express a protein binding to the protein of the invention is applied to the column. As this test sample, a cell culture supernatant, cell extract, cell lysate, and such may be used. After applying the test sample, the column is washed to obtain the protein binding to the protein of the invention.

[0103]

The compound isolated by the screening method of the invention is a candidate drug for promoting or inhibiting the activity of the protein of the invention. The compound obtained by using the screening method of the invention encompasses a compound resulting from modifying the compound having an activity to bind to the protein of the invention by adding, deleting, and/or replacing a part of the structure.

[0104]

When using the compound obtained by the screening method of the invention as drugs for humans and mammals such as, mice, rats, guinea pigs, rabbits, chicken, cats, dogs, sheep, pigs, cattle, monkeys,

sacred baboons, and chimpanzees, the drug may be administered using ordinary means.

[0105]

For example, according to the need, the drugs can be taken orally as sugar-coated tablets, capsules, elixirs, and microcapsules, or parenterally in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds comprising the activity to bind to the protein of the invention can be mixed with physiologically acceptable carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, and binders, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

[0106]

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum, and arabic gum; excipients such as crystalline cellulose; swelling agents such as cornstarch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose, or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil, and cherry. When the unit dosage form is a capsule, a liquid carrier, such as oil, can also be included in the above additives. Sterile compositions for injections can be formulated following usual drug implementations using vehicles such as distilled water used for injections.

[0107]

For example, physiological saline and isotonic liquids including glucose or other adjuvants, such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

[0108]

Sesame oil or soy-bean oil can be used as a oleaginous liquid

and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer; may be formulated with a buffer such as phosphate buffer and sodium acetate buffer; a pain-killer such as procaine hydrochloride; a stabilizer such as benzyl alcohol and phenol; and an anti-oxidant. The prepared injection is usually filled into a suitable ampule.

[0109]

Although the dosage of the compound that has the activity to bind to the protein of the invention varies according to symptoms, the daily dose is generally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg, when administered orally to an adult (body weight 60 kg).

[0110]

When given parenterally, the dose differs according to the patient, target organ, symptoms, and method of administration, but the daily dose is usually about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg and more preferably about 0.1 to about 10 mg for an adult (body weight 60 kg) when given as an intravenous injection. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kg of body-weight.

[0111]

The antibody of the present invention can be obtained as a monoclonal antibody or a polyclonal antibody using well-known methods.

[0112]

The antibody that specifically binds to the protein of the invention can be prepared by using the protein of the invention as a sensitizing antigen for immunization according to usual immunizing methods, fusing the obtained immunized cells with known parent cells by ordinary cell fusion methods, and screening for antibody producing cells using the usual screening techniques.

[0113]

Specifically, a monoclonal or polyclonal antibody that binds to the proteins of the invention may be prepared as follows.

[0114]

For example, the protein of the invention that is used as a sensitizing antigen for obtaining the antibody is not restricted by

the animal species from which it is derived, but is preferably a protein derived from mammals, for example, humans, mice, or rats, especially from humans. Proteins of human origin can be obtained by using the nucleotide sequence or amino acid sequence disclosed herein.

[0115]

The protein that is used as a sensitizing antigen in the present invention can be a protein that comprises the biological activity of all the proteins described herein. Partial peptides of the proteins may also be used. As partial peptides of the proteins, for example, the amino (N) terminal fragment of the protein, and the carboxy (C) terminal fragment can be given. "Antibody" as used herein means an antibody that specifically reacts with the full-length or fragment of the protein.

[0116]

A gene encoding the protein of the invention or a fragment thereof is inserted into a well-known expression vector, and after transforming the host cells described herein, the objective protein or a fragment thereof is obtained from within and without the host cell, or from the host using well-known methods, and this protein can be used as a sensitizing antigen. Also, cells expressing the protein, cell lysates, or chemically synthesized protein of the invention may be used as a sensitizing antigen.

[0117]

The mammals that are immunized by the sensitizing antigen are not restricted, but it is preferable to select the animal by considering the adaptability with the parent cells used in cell fusion. Generally, an animal belonging to Rodentia, Lagomorpha, or Primates is used.

[0118]

As animals belonging to Rodentia, for example, mice, rats, hamsters, and such are used. As animals belonging to Lagomorpha, for example rabbits, as Primates, for example monkeys, are used. As monkeys, monkeys of the infraorder Catarrhini (Old World Monkeys), for example, cynomolgus monkeys, rhesus monkeys, sacred baboons, chimpanzees, etc., are used.

[0119]

To immunize animals with the sensitizing antigen, well-known

methods may be used. For example, the sensitizing antigen is generally injected into mammals intraperitoneally or subcutaneously. Specifically, the sensitizing antigen is suitably diluted, suspended in physiological saline or phosphate-buffered saline (PBS), mixed with a suitable amount of a general adjuvant if desired, for example, with Freund's complete adjuvant, emulsified and injected into the mammal. Thereafter, the sensitizing antigen suitably mixed with Freund's incomplete adjuvant is preferably given several times every four to 21 days. A suitable carrier can also be used when immunizing an animal with the sensitizing antigen. After the immunization, the elevation in the serum antibody level is detected by usual methods.

[0120]

Polyclonal antibodies against the protein of the invention can be obtained as follows. After verifying that the desired serum antibody level has been reached, blood is withdrawn from the mammal sensitized with the antigen. Serum is isolated from this blood using well-known methods. The serum containing the polyclonal antibody may be used as the polyclonal antibody, or according to needs, the polyclonal antibody-containing fraction may be further isolated from the serum.

[0121]

To obtain monoclonal antibodies, after verifying that the desired serum antibody level has been reached in the mammal sensitized with the above-described antigen, immunocytes are taken from the mammal and used for cell fusion. At this instance, immunocytes that are preferably used for cell fusion are splenocytes. As parent cells fused with the above immunocytes, preferable are mammalian myeloma cells, more preferable are, myeloma cells that have attained the feature of distinguishing fusion cells by agents.

[0122]

For the cell fusion between the above immunocytes and myeloma cells, for example, the method of Milstein et al. (Galfre, G. and Milstein, C., Methods Enzymol., 1981, 73, 3-46) is basically well known.

[0123]

The hybridoma obtained from cell fusion is selected by culturing in a usual selective culture medium, for example, HAT culture medium (hypoxanthine, aminopterin, thymidine-containing culture medium).

The culture in this HAT medium is continued for a period sufficient enough for cells (non-fusion cells) other than the objective hybridoma to perish, usually from a few days to a few weeks. Next, the usual limiting dilution method is carried out, and the hybridoma producing the objective antibody is screened and cloned.

[0124]

Other than the above method of obtaining a hybridoma by immunizing an animal other than humans with the antigen, a hybridoma producing the objective human antibodies comprising the activity to bind to proteins can be obtained by the method of sensitizing human lymphocytes, for example, human lymphocytes infected with the EB virus, with proteins, protein-expressing cells, or lysates thereof *in vitro*, fusing the sensitized lymphocytes with myeloma cells derived from human, for example U266, having the capacity of permanent cell division (Unexamined Published Japanese Patent Application (JP-A) No. Sho 63-17688).

[0125]

Moreover, human antibody against the protein can be obtained using a hybridoma made by fusing myeloma cells with antibody-producing cells obtained by immunizing a transgenic animal comprising a repertoire of human antibody genes with an antigen such as a protein, protein-expressing cells, or a cell lysate thereof WO92/03918, WO93/2227, WO94/02602, WO94/25585, WO96/33735, and WO96/34096).

[0126]

Other than producing antibodies by using hybridoma, antibody-producing immunocytes such as sensitized lymphocytes that are immortalized by oncogenes may also be used.

[0127]

Such monoclonal antibodies can also be obtained as recombinant antibodies produced by using the genetic engineering technique (for example, Borrebaeck, C.A.K. and Larrick, J.W., THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990). Recombinant antibodies are produced by cloning the encoding DNA from immunocytes such as hybridoma or antibody-producing sensitized lymphocytes, incorporating this into a suitable vector, and introducing this vector into a host to produce the antibody. The

present invention encompasses such recombinant antibodies as well.

[0128]

The antibody of the present invention may be an antibody fragment or a modified-antibody as long as it binds to the protein of the invention. For example, Fab, $F(ab')_2$, Fv, or single chain Fv in which the H chain Fv and the L chain Fv are suitably linked by a linker (scFv, Huston, J.S. et al., Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 5879-5883) can be given as antibody fragments. Specifically, antibody fragments are produced by treating an antibody with an enzyme, for example, papain, pepsin, etc. or by constructing a gene encoding an antibody fragment, introducing this into an expression vector, and expressing this vector on suitable host cells (for example, Co, M.S. et al., J. Immunol., 1994, 152, 2968-2976; Better, M. and Horwitz, A.H., Methods Enzymol., 1989, 178, 476-496; Pluckthun, A. and Skerra, A., Methods Enzymol., 1989, 178, 497-515; Lamoyi, E., Methods Enzymol., 1986, 121, 652-663; Rousseaux, J. et al., Methods Enzymol., 1986, 121, 663-669; Bird, R.E. and Walker, B.W., Trends Biotechnol., 1991, 9, 132-137).

[0129]

As a modified antibody, an antibody bound to various molecules such as polyethylene glycol (PEG) can be used. The present antibody encompasses such modified antibodies as well. To obtain such a modified antibody, chemical modifications are done to the obtained antibody. These methods are already established in the field.

[0130]

The antibody of the invention may be obtained as a chimeric antibody comprising non-human antibody-derived variable region and a human antibody-derived constant region, or as a humanized antibody comprising non-human antibody-derived complementarity determining region (CDR), and human antibody-derived framework region (FR) and a constant region.

[0131]

Antibodies thus obtained can be purified till uniform. The separation and purification methods for separating and purifying the antibody used in the present invention may be any method usually used for proteins, and is not in the least limited. Antibody concentration of the above mentioned antibody can be assayed by measuring the

absorbance, or by the enzyme-linked immunosorbent assay (ELISA), etc.

[0132]

Also, as methods that assay the antigen-binding activity of the antibody of the invention, ELISA, enzyme immunoassay (EIA), radio immunoassay (RIA), or fluorescent antibody method can be given. For example, when using ELISA, the protein of the invention is added to a plate coated with the antibody of the invention, and next, the objective antibody sample, for example, culture supernatants of antibody-producing cells, or purified antibodies are added. Then, secondary antibody recognizing the antibody, which is labeled by alkaline phosphatase and such enzymes, is added, the plate is incubated and washed, and absorbance is measured to evaluate the antigen-binding activity after adding an enzyme substrate such as p-nitrophenyl phosphate. As the protein, a protein fragment, for example, a fragment comprising a C terminus, or a fragment comprising an N terminus may be used. To evaluate the activity of the antibody of the invention, BIAcore (Pharmacia) may be used.

[0133]

By using these methods, the antibody of the invention and a sample presumed to contain the protein of the invention are contacted, and the protein of the invention is detected or assayed by detecting or assaying the immune complex of the above-mentioned antibody and protein.

[0134]

A method of detecting or assaying the protein of the invention is useful in various experiments using proteins as it can specifically detect or assay the proteins.

[0135]

The present invention also encompasses a DNA specifically hybridizing to a DNA comprising a nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, and 8, or its complementary DNA, and comprising at least 15 nucleotides. Namely, a probe that can selectively hybridize to the DNA encoding the protein of the invention, or a DNA complementary to the above DNA, a nucleotide or nucleotide derivative, for example, antisense oligonucleotide, ribozyme, and such are included.

[0136]

The present invention also encompasses an antisense oligonucleotide that hybridizes to any portion of any one of the nucleotide sequences shown in, for example, SEQ ID NOs: 2, 4, 6, and 8. This antisense oligonucleotide is preferably one against at least 15 continuous nucleotides in any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, and 8. More preferable is the above-mentioned antisense oligonucleotide against the above-mentioned at least 15 continuous nucleotides containing a translation start codon.

[0137]

Derivatives or modified products of antisense oligonucleotides can be used as antisense oligonucleotides. As such modified products, for example, lower alkyl phosphonate modifications such as methyl-phosphonate-type or ethyl-phosphonate-type, phosphorothioate or phosphoroamidate-modified products, etc. may be used.

[0138]

The term "antisense oligonucleotide(s)" as used herein means, not only those in which the nucleotides corresponding to those constituting a specified region of a DNA or mRNA are entirely complementary, but also those having a mismatch of one or more nucleotides, as long as the DNA or mRNA and the oligonucleotide can selectively and stably hybridize with the nucleotide sequence of SEQ ID NO: 1.

[0139]

"Selectively and stably hybridize" means that significant cross hybridization with DNA encoding other proteins does not occur under usual hybridization conditions, preferably under stringent hybridization conditions. Such DNAs are indicated as those having, in the "at least 15 continuous nucleotide" sequence region, a homology of at least 70% or higher, preferably 80% or higher, more preferably 90% or higher, even more preferably 95% or higher nucleotide sequence homology. The algorithm stated herein can be used to determine homology. Such DNA is useful as a probe for detecting or isolating DNA encoding the protein of the invention, or as a primer for amplification as described in Examples below.

[0140]

The antisense oligonucleotide derivative of the present invention acts upon cells producing the protein of the invention by binding to the DNA or mRNA encoding the protein to inhibit its transcription or translation, and to promote the degradation of mRNA, and has an effect of suppressing the function of the protein of the invention by suppressing the expression of the protein.

[0141]

The antisense oligonucleotide derivative of the present invention can be made into an external preparation such as a liniment and a poultice by mixing with a suitable base material, which is inactive against the derivatives.

[0142]

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops, and freeze-dried agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, etc. These can be prepared using the usual methods.

[0143]

The antisense oligonucleotide derivative is given to the patient by directly applying onto the ailing site, by injecting into a blood vessel, etc. so that it will reach the ailing site. An antisense-mounting material can also be used to increase durability and membrane-permeability. Examples are, liposome, poly-L lysine, lipid, cholesterol, lipofectin, or derivatives of these.

[0144]

The dosage of the antisense oligonucleotide derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

[0145]

The antisense oligonucleotide derivative of the present invention is useful in inhibiting the expression of the protein of the invention, and therefore is useful in suppressing the biological activity of the protein of the invention. Also, expression-inhibitors comprising the antisense oligonucleotide derivative of the present invention are useful because of their capability to suppress the biological

activity of the protein of the invention.

[0146]

[Examples]

The present invention shall be described in detail below with reference to examples, but is not be construed as being limited thereto.

[0147]

[Example 1] Two step Blast Search

Probe sequences (256 types) comprising the tggag(t/c)nnntggag(t/c) (where n is an arbitrary nucleotide) as the oligonucleotide encoding the Trp-Ser-Xaa-Trp-Ser motif were designed. These sequences enable the detection of almost all known hemopoietin receptors, except for the EPO receptor, TPO receptor, and the mouse IL6 receptor. Using each sequence as the query, the GenBank nr database was searched using the BlastN (Advanced BlastN 2.0.4) program. Default values (Descriptions=100, Alignments=100) were used as parameters for the search, except for making the expectation value 100.

[0148]

Since approximately 500 clones that completely matched the probe sequences were obtained as a result of the primary search, among these, a 180-residue nucleotide sequence of human genome-derived clones (cosmid, BAC, and PAC) containing the probe sequence in approximately the center was excised. Next, using this 180-residue nucleotide sequence as the query, the nr database was searched again using the BlastX (Advanced BlastX 2.0.4) program to search the homology of the amino acid sequence around the probe sequence with known hemopoietin receptors.

[0149]

Default values were used as parameters for the search, except for making the expectation value 100. However, when extremely large number of hits were obtained (caused by the Alu sub family that is a high repetitive sequence), it was often difficult to observe hits for known hemopoietic receptors. Therefore, to maximize the sensitivity in such cases, a value of "Expect=1000, Descriptions=500, Alignments=500" was used.

[0150]

As a result of the secondary search by BlastX, 28 clones hit one

or more known hemopoietin receptors (Table 1 to Table 8).

[0151]

[Table 1]

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGTAATTGGAGC	Asn	AL009181	30692 tggagtaattggagc 30678	1p34.1-1p35	mIL11R(opposite), FOBR
TGGAGCTGATGGAGC	***	Z97987	140006 tggagctgatggagc 139992	1p36.2-36.3	line1, Leu Zip p40,
TGGAGCAGCTGGAGC	Ser	AF023268	39931 tggagcagctggagc 39917	1q21	metaxin
TGGAGCTGCTGGAGC	Cys	AL009051	78023 tggagctgctggagc 78037	1q23-24	HP-10, semaphorin F,G
TGGAGCACGTGGAGT	Thr	Z97200	112905 tggagcacgtggagt 112891	1q24	AFP enhancer BP, RAR
TGGAGTGCCTGGAGC	Ala	U95626	101031 tggagtgcctggagc 101017	3	CFTC, TcR
TGGAGTAGATGGAGT	Arg	Z84495	2547 tggagtagatggagt 2533	3p21.3	trithorax
TGGAGCTGATGGAGT	***	Z74023	5255 tggagctgatggagt 5241	3p21.3	E2ABP, fibronectin, nidgen
TGGAGTTTCTGGAGT	Phe	Z68275	7291 tggagtttctggagt 7277	4p16.3	mena, NMDAR
TGGAGTGCCTGGAGT	Ala	Z54072	21277 tggagtgcctggagt 21291	4p16.3	crk, AchR, HER3
TGGAGCTGCTGGAGC	Cys	Z69837	30266 tggagctgctggagc 30252	4p16.3	KIT, FLT3, PDGFRa
TGGAGTTACTGGAGT	Tyr	AC003951	27290 tggagttactggagt 27304	5	collagen
TGGAGCCTGTGGAGT	Leu	AC004502	48334 tggagcctgtggagt 48320	5	ADAMTS-1, properdin, etc
TGGAGTTGATGGAGC	***	L81613	2418 tggagttgatggagc 2404	5	APC, bat2, p53
TGGAGTGTATGGAGT	Val	AC002122	43679 tggagtgtatggagt 43665	5p15.2	Met tRNA syntase
TGGAGTCCATGGAGT	Pro	AC002380	34646 tggagtccatggagt 34632	5p15.2	N-WASP, enigma
TGGAGCAACTGGAGC	Asn	AC002479	80443 tggagcaactggagc 80457	5p15.2	NEU, glycoprotein C
TGGAGCTGCTGGAGT	Cys	AC004592	125445 tggagctgctggagt 125431	5q31	CD22-B
TGGAGTAGCTGGAGT	Ser	AC002393	3721 tggagtagctggagt 3735	6	glycoprotein
TGGAGTTGCTGGAGT	Cys	AC002326	114578 tggagttgctggagt 114564	6	G3P REGULON
TGGAGTGCATGGAGT	Ala	Z84490	20244 tggagtgcatggagt 20230	6	Alu, adrenergic receptor

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Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TTGGAGTTTCTGGAGC	Phe	AC002112	68699 tggagtttctggagc 68685	6	IgHv, MYD116
TGGAGCGGCTGGAGC	Gly	U89336	35829 tggagcggctggagc 35815	6p21	myosin HC, cep250,
TGGAGCGTCTGGAGC	Val	U53588	3558 tggagcgtctggagc 3572	6p21.3	ring finger, BRCA1
TGGAGTGCATGGAGT	Ala	Z98744	38358 tggagtgcatggagt 38344	6p21.3-22.3	Alu, AD7c-NTP
TGGAGTTGCTGGAGT	Cys	AL009031	104325 tggagttgctggagt 104311	6p22.3-24.1	ACC synthase
TGGAGTGTCTGGAGT	Val	AL008729	21325 tggagtgtctggagt 21339	6p24	E1A, DUB-2
TGGAGTTGTTGGAGT	Cys	Z98755	69825 tggagttgttggagt 69811	6q16.1-21	dynein
TGGAGCTTCTGGAGC	Phe	Z98172	35554 tggagcttctggagc 35540	6q21	HGXPRT
TGGAGCAGGTGGAGC	Arg	Z97989	79116 tggagcaggtggagc 79102	6q21-22	syn fyn, slk, yes, src
TGGAGCTAATGGAGT	***	Z95326	16562 tggagctaatggagt 16576	6q22.1-6q22.33	tyrosinase
TGGAGCTCTTGGAGC	Ser	Z98049	25800 tggagctcttggagc 25786	6q26-q27	collagen, AT3, C1Qb
TGGAGCTCCTGGAGT	Ser	AC003090	22068 tggagctcctggagt 22082	7p15	ICE
TGGAGTATATGGAGC	Ile	AC004744	22740 tggagtatatggagc 22754	7p15-p21	TSH-R, RNABP
TGGAGTAGCTGGAGC	Ser	AC004485	86356 tggagtagctggagc 86370	7p15-p21	Hox 2.4, mIL11Ra(stop*)
TGGAGTCTTTGGAGT	Leu	AC004141	3130 tggagtctttggagt 3144	7p21-p22	polyprotein
TGGAGCAGATGGAGC	Arg	AC004548	62876 tggagcagatggagc 62862	7q11.23-q21.1	NCAM
TGGAGCAACTGGAGT	Asn	AC002456	69500 tggagcaactggagt 69514	7q21	glycoprotein A
TGGAGTAACTGGAGT	Asn	AC000064	9170 tggagtaactggagt 9184	7q21-22	GA3PD
TGGAGTTATTGGAGT	Tyr	AC003085	87341 tggagttattggagt 87355	7q21-22	Nmyc, FGFR
TGGAGTTGTTGGAGT	Cys	AC000119	65235 tggagttgttggagt 65221	7q21-7q22	FVIII, TopoIII
TGGAGTTGTTGGAGT	Cys	AC002458	44435 tggagttgttggagt 44421	7q21-q22	telomerase, NFAT
TGGAGTACATGGAGC	Thr	AC000059	9977 tggagtacatggagc 9963	7q21-7q22	Alu, Notch4

[Table 3]

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGTCAGTCGAGC	Gln	AC000119	103655 tggagtcagtcgagc 103669	7q21-7q22	reverse transcriptase
TTGGAGTATTTGGAGT	Ile	AC002384	52216 tggagtatttggagt 52202	7q22	pol, GHR(another frame)
TGGAGCAGCTGGAGT	Ser	AC004522	55291 tggagcagctggagt 55277	7q22-q31.1	hemoglobin beta
TGGAGTGTTTGGAGT	Val	AC002466	43273 tggagtgtttggagt 43287	7q31	ryanodine receptor、mTPO
TGGAGTGGCTGGAGC	Gly	AC002543	112948 tggagtggctggagc 112962	7q31.2	EGF, P-selectin
TGGAGCTGATGGAGC	***	AC000061	79564 tggagctgatggagc 79550	7q31.2	laminin B1, tubulin
TGGAGTTTTTGGAGT	Phe	AC000125	13750 tggagttttggagt 13736	7q31.3	p150
TGGAGTTGTTGGAGT	Cys	AC002498	20166 tggagttgttggagt 20152	7q31.3	IL3Rb(opposite)
TGGAGCGGGTGGAGC	Gly	U66059	158491 tggagcgggtggagc 158477	7q35(TcRb)	properdin
TGGAGCATTTGGAGC	Ile	AC003109	4761 tggagcatttggagc 4775	7q36	CD2, HOX-2.6
TGGAGTTATTGGAGT	Tyr	AF027390	174448 tggagttattggagt 174434	7q tel	IkB, V2R
TGGAGCATATGGAGT	Ile	AC002052	28882 tggagcatatggagt 28896	9p22	myosin VIIA, OSMRb
TGGAGCAACTGGAGT	Asn	AC001643	27345 tggagcaactggagt 27331	9q34	hox1.4, gastrinR
TGGAGCAACTGGAGT	Asn	AC002319	87518 tggagcaactggagt 87532	9q34	hox1.4, gastrinR
TGGAGCGGATGGAGC	Gly	AC000396	16394 tggagcggatggagc 16380	9q34	vWf, laminin a3
TGGAGTGAGTGGAGT	Glu	U73649	16850 tggagtgagtggagt 16836	11	zinc finger
TGGAGTGGGTGGAGT	Gly	U73649	16859 tggagtgggtggagt 16845	11	zinc finger
TGGAGTGCCTGGAGT	Ala	U73629	31027 tggagtgcctggagt 31041	11	Alu, gp2b, BCGF-12
TGGAGCCCCTGGAGT	Pro	U73629	36731 tggagccccctggagt 36745	11	EZRIN, hemagglutinin
TGGAGTCCCTGGAGC	Pro	U73643	14550 tggagtccctggagc 14564	11	reverse transcriptase
TGGAGCAACTGGAGC	Asn	AF015116	65621 tggagcaactggagc 65635	11p15.5	Nasopressin R, OSMR
TGGAGTGCATGGAGT	Ala	AC002350	23543 tggagtgcatggagt 23529	12q24	Alu, IFNaR

[0154]

[Table 4]

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGTGCATGGAGT	Ala	AC004217	88822 tggagtgcatggagt 88808	12q24.1	Alu, HPK
TTGGAGTTACTGGAGC	Tyr	AC002978	65893 tggagttactggagc 65907	12q24	clathrin LC, EPOR(nonWS)
TGGAGTTGTTGGAGT	Cys	AC000403	91715 tggagttgttggagt 91729	13	VHL, inhibin B
TGGAGCGGTTGGAGC	Gly	X97051	73621 tggagcggttggagc 73607	14q32.33 (IgD)	polycystic kidney
TGGAGTAGGTGGAGC	Arg	AC003024	15596 tggagtaggtggagc 15582	15q26	pksF
TGGAGTTTCTGGAGC	Phe	AC002102	93356 tggagtttctggagc 93370	16	pol, PHLN
TGGAGTTCATGGAGT	Ser	U91318	102406 tggagttcatggagt 102392	16	ICAM1, MIBP1
TGGAGTGTATGGAGT	Val	AC002289	10631 tggagtgtatggagt 10645	16	Alu
TGGAGCCACTGGAGT	His	U91318	15252 tggagccactggagt 15238	16	laminin alpha1
TGGAGTTAATGGAGT	***	AC002519	81768 tggagttaatggagt 81754	16	Rho, Notch
TGGAGCTGCTGGAGT	Cys	U91326	84127 tggagctgctggagt 84113	16p11.2	NIPI-like, IL2Rz(nonWS)
TGGAGTGAATGGAGT	Glu	AC002303	100952 tggagtgaatggagt 100966	16p12	TFDR, OBR, and many
TGGAGCACTTGGAGC	Thr	AC002551	82245 tggagcacttggagc 82259	16p12.1	envelope, androgen R
TGGAGTCCCTGGAGC	Pro	AC002299	162 tggagtccctggagc 148	16p12-p13.1	CYCLIN H, FN
TGGAGCTATTGGAGC	Tyr	AC002299	84540 tggagctattggagc 84526	16p12.p13.1	AUF, RNA editase
TGGAGTCACTGGAGT	His	U95737	16130 tggagtcactggagt 16144	16p13.1	TcRa, HLAa
			16374 tggagtcactggagt 16388		Notch, Pro-rich
			16599 tggagtcactggagt 16613		phosphatase, ORFB
TGGAGTCCTTGGAGG	Pro	U91318	11227 tggagccttggagc 11286	16p18.11	CD30, collagen, MAP1a
TGGAGCACTTGGAGC	Thr	AC004609	26031 tggagcacttggagc 26045	16p13.3	TcRb
TGGAGCCGTTGGAGC	Arg	AC004496	28217 tggagccgttggagc 28231	16p13.3	mucin, ET1, IL12R(nonWS)

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGCCGCTGGAGC	Arg	AC004232	34550 tggagccgctggagc 34564	16p13.3	IgLk, AGPR
TTGGAGTACTTGGAGC	Thr	AJ003147	151180 tggagtacttggagc 151166	16p13.3	RanBP2
TGGAGCGTGTGGAGC	Val	X71874	11520 tggagcgtgtggagc 11534	16q22.1	collagen a5IV
TGGAGCAAATGGAGT	Lys	AC003663	114346 tggagcaaatggagt 114360	17	beta-D-glucosidase
TGGAGTCTCTGGAGC	Leu	AC003957	52898 tggagtctctggagc 52884	17	TIE-1, SEX, Rho,
TGGAGCAGATGGAGC	Arg	AC003971	76277 tggagcagatggagc 76263	18	LIMK-1, TcR
TGGAGTGCATGGAGT	Ala	AD000812	30891 tggagtgcatggagt 30905	19	Alu
[illegible]	Ala	AC002126	85832 tggagtgcatggagt 85846	19	Alu, AD7c-NTP
TGGAGCTGCTGGAGT	Cys	AC004660	10008 tggagctgctggagt 10022	19	Reps1
TGGAGCCCCTGGAGT	Pro	AC004490	14389 tggagcccctggagt 14403	19	mucin, ataxin-2, N-WASP
TGGAGTGAGTGGAGC	Glu	AC003112	18315 tggagtgagtggagc 18301	19p12(NIR6)	TPOR, PRLR, OBR, etc
TGGAGCAGATGGAGC	Arg	AC004004	39010 tggagcagatggagc 38996	19p12	PRLR, IL12R, GM-CSFRb, IL11R(+stop codon)
		-----presumably a pseudogene-----			
			39177 tggagcagatggagc 39163		IL3Ra(weak, 22 nonWS)
TGGAGCACCTGGAGT	Thr	AD000685	21015 tggagcacctggagt 21001	19p13.1	GM-CSFRb(nonWS+stop)
TGGAGCTGATGGAGC	***	AC002115	37164 tggagctgatggagc 37178	19q13.1	Mpc2, Pro rich protein
TGGAGCCAGTGGAGC	Gln	M63796	7622 tggagccagtggagc 7636	19q13.3	NFCP, titin, Jagged 2
TGGAGTTACTGGAGT	Tyr	AC004505	31711 tggagttactggagt 31725	20	Gap junction
TGGAGTTGATGGAGC	***	Z93016	31093 tggagttgatggagc 31079	20q12-13.2	smaphorin F, GHS-R, JAK2
TGGAGTCAATGGAGT	Gln	L35677	579 tggagtcaatggagt 565	21(MX1)	GLI, IL9R, IL7R(nonWS)
TGGAGTGCCTGGAGT	Ala	AF039907	29892 tggagtgcctggagt 29906	21	IgV, Cyt.Oxidase
TGGAGTGTCTGGAGT	Val	AG000937	105 tggagtgtctggagt 91	21q	peroxidasin

[0156]

[Table 6]

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGTAAATGGAGT	Lys	AP000034	28803 tggagtaaatggagt 28789	21q11.1	Na/Ca exchanger
TTGGAGTAGGTGGAGT	Arg	AP000039	24900 tggagtagctggagt 24914	21q11.1	RNA polymerase
TGGAGTGAGTGGAGT	Glu	AP000035	21721 tggagtgagtggagt 21707	21q11.1	smaphorin F
TGGAGTGTCTGGAGT	Val	AG000038	26164 tggagtgtctggagt 26150	21q11.1	Glycoprotein
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TGGAGTGCCTGGAGT	Ala	AP000045	7204 tggagtgcctggagt 7218	21q11.1	IgV,
TGGAGCATTTGGAGC	Ile	AP000052	93726 tggagcatttggagc 93740	21q11.1	Ig H, TCF-3, CETP
TGGAGCCTCTGGAGC	Leu	AP000037	17581 tggagcctctggagc 17567	21q11.1	Alu, BCGF
TGGAGTGGGTGGAGT	Gly	AP000015	48480 tggagtgggtggagt 48494	21q22.2	TPO
TGGAGTGAGTGGAGT	Glu	Z97065	151632 tggagtgagtggagt 151618	22	semaphorin H, CD44
TGGAGCTGGTGGAGT	Trp	Z83856	8503 tggagctggtggagt 8489	22	ERF
TGGAGTGGGTGGAGT	Gly	Z95113	69325 tggagtgggtggagt 69311	22q11.2-qter	factor H
TGGAGTGCATGGAGT	Ala	Z93784	36348 tggagtgcatggagt 36362	22q11.2-qter	Alu, NF2
TGGAGCCTCTGGAGT	Leu	AC002308	130741 tggagcctctggagt 130727	22q11.2	collagen a1, Na channel
TGGAGTCCCTGGAGC	Pro	AC000086	40705 tggagtccctggagc 40691	22q11.2	ADH, collagen
TGGAGCATCTGGAGC	Ile	L77569	21088 tggagcatctggagc 21074	22q11DiGeorgeclathrin heavy chain 2	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TGGAGCAGCTGGAGC	Ser	AC000092	9817 tggagcagctggagc 9803	22q11.2	IgHv, PC binding
TGGAGCAACTGGAGC	Asn	Z95116	64481 tggagcaactggagc 64495	22q12.1	p150, IL4RQWSNWSF*)
TGGAGCTAGTGGAGC	***	AC003071	114780 tggagctagtggagc 114794	22q12.1-qter	FGFRb
TGGAGCCCTTGGAGC	Pro	Z80902	2675 tggagcccttggagc 2661	22q12-qter	collagen a1
TGGAGCTCTTGGAGT	Ser	Z79999	40825 tggagctcttggagt 40839	22q12-qter	collagen a1,

[0157]

[Table 7]

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGCCATTGGAGT	His	Z81308	12676 tggagccattggagt 12661	22q12-qter	MYF-6, p53, INK4a
TGGAGTGCAGCTTGGAGT	Glu	AL008637	85322 tggagtgcagcttggagt 85338	22q12.3-13.2	GM-CSFRb, IL3RA/EPOR, etc
TTGGAGTGAGTGGAGT	Glu	U62317	77740 tggagtgagtggagt 77726	22q13	latrophilin-related
TGGAGTGCATGGAGT	Ala	Z82018	31082 tggagtgcatggagt 31068	22q13	Alu, G-GSTM, AD7c-NTP
TGGAGTTGTTGGAGT	Cys	AC002422	19151 tggagttgttggagt 19137	X	cGMP PDase
TGGAGTGTCTGGAGT	Val	Z73418	31830 tggagtgtctggagt 31816	X	WNT-8D, Mi-2
TGGAGTCTTTGGAGT	Leu	Z83843	114972 tggagtctttggagt 114958	X	reverse transcriptase
TGGAGTCTCTGGAGT	Leu	Z99706	7749 tggagtctctggagt 7735	X*	Selenoprotein
TGGAGCAACTGGAGT	Asn	AC002420	70704 tggagcaactggagt 70690	X	homeoprotein, ΩBR(stop)
TGGAGCATGTGGAGT	Met	Z77210	5702 tggagcatgtggagt 5688	X	TcRb, CLIP-1
TGGAGTTCCTGGAGC	Ser	Z83131	4904 tggagttcctggagc 4890	X	VPS41 homolog
TGGAGTGGCTGGAGC	Gly	AC004388	239975 tggagtggctggagc 239989	X	GAP, mLIFR(stop)
TGGAGTGGCTGGAGC	Gly	AC004478	73509 tggagtggctggagc 73495	X	RNAse, mLIFR(stop)
TGGAGTCTATGGAGC	Leu	Z70050	9934 tggagtctatggagc 9948	X	complement C8, C7
TGGAGTCTATGGAGC	Leu	Z73986	40766 tggagtctatggagc 40780	X	complement C8, C7
TGGAGCTGTTGGAGC	Cys	L44140	112657 tggagctgttggagc 112671	X	rab GDI alpha, BDGF
TGGAGCTCATGGAGC	Ser	AC004383	144906 tggagctcatggagc 144892	X	RTase, transposon
TGGAGTAAATGGAGC	Lys	Z69732	31681 tggagtaaatggagc 31695	Xp11	OT-R, acrosin
TGGAGTTCGTGGAGC	Ser	Z92545	88703 tggagttcgtggagc 88717	Xp11	PMK1
TGGAGCTTCTGGAGC	Phe	AL008709	46089 tggagcttctggagc 46075	Xp11.23-Xp11.4	rMHC class 1a, HLA-C
TGGAGTTTCTGGAGT	Phe	U96409	116332 tggagtttctggagt 116346	Xp22	myosin H
TGGAGTTGCTGGAGT	Cys	AC000110	89544 tggagttgctggagt 89530	Xp22	IL2R

Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TTGGAGTCACTGGAGT	His	AL021706	11982 tggagtcactggagt 11968	Xq21.1-21.33	dopamine receptor
TGGAGCTGGTGGAGT	Trp	AC000113	119188 tggagctggtggagt 119202	Xq23	DNA repair protein, MHC
TGGAGCAAGTGGAGT	Lys	AF007262	98212 tggagcaagtggagt 98226	Xq28	RNA polymerase
TGGAGCTGCTGGAGT	Cys	U82671	35792 tggagctgctggagt 35806	Xq28	XTCF-3c
TGGAGTCAGTGGAGC	Gln	AF011889	144465 tggagtcagtggagc 144451	Xq28	GHRHR, Werner Synd.
TGGAGCTAATGGAGC	***	AF030876	107409 tggagctaatggagc 107395	Xq28	gp41, clk3
TGGAGCTAATGGAGC	***	AF031078	94438 tggagctaatggagc 94424	Xq28	gp41
TGGAGCTAATGGAGC	***	Z47046	15688 tggagctaatggagc 5624	Xq28	gp41
TGGAGTTTCTGGAGT	Phe	AC002531	106698 tggagtttctggagt 106712	Y	Alu, hpk
TGGAGCAGTTGGAGC	Ser	AC004474	124745 tggagcagttggagc 124731	Y	EGFR, Smad6
TGGAGTTTGTGGAGT	Leu	U26425	12899 tggagtttgtggagt 12913	PLCb2	PRLR(opposite)
TGGAGCAACTGGAGT	Asn	U96726	61672 tggagcaactggagt 61658	mouse DNA	envelope mIL11R(opposite)
TGGAGTCCCTGGAGC	Pro	U35928	22244 tggagtccctggagc 22230	MHC class II	CFTC, IL6RI
TGGAGCAGATGGAGC	Arg	AC002482	14276 tggagcagatggagc 14290	RG208O03	I-309, TcR, IL9R(nonWS)
TGGAGCTCTTGGAGC	Ser	U34879	24914 tggagctcttggagc 24928	EDH17B2	Large tegument protein commonB(oppsit. nonWS)
TGGAGCCTTTGGAGC	Leu	Z15025	6359 tggagcctttggagc 6373	Bat2	bat2,mucin, GM-CSFRb(opposite, stop)

Redundant clones are shadowed. White and underlined letters indicate hits and pseudo-hits, respectively.

[0159]

Four clones out of these 28 clones (AC002303, AC003112, AL008637, and AC004004) hit several known hemopoietin receptors, however, AC004004 was excluded as it has a stop codon downstream three amino acids of the Trp-Ser-Xaa-Trp-Ser motif. Among the three remaining clones, AL008637 was thought to be a known receptor, GM-CSF receptor β. AC002303 is the BAC clone CIT987-SKA-670B5 derived from the 16p12 region of human chromosome no. 16 registered by TIGR group on June 19, 1997 and comprises the full-length of 131530 base pairs (Lamerdin, J.E., et al., GenBank Report on AC003112, 1997).

[0160]

As shown in Fig. 1, a BlastX search (query: 180 nucleotides of 40861-41040 including tggagtgaatggagt (40952-40966), the only probe sequence within the AC002303) revealed that numerous hemopoietin receptors starting with the TPO receptor and leptin receptor show an evident homology, however, there were no known, database-registered hemopoietin receptors that completely matched the query sequence. Also, a BlastX scanning was done under the above conditions, by excising a sequential 180-residue nucleotide sequence in both the 5′ and 3′ directions, centering on the 180-residue nucleotide sequence mentioned above, and when this was used as a query, two sequences having a homology to known hemopoietin receptors were found in the regions 39181-39360 and 42301-42480, and were thought to be other exons of the same gene (Fig. 2).

[0161]

A Pro-rich motif PAPPF was conserved in the 39181-39360 site, and a Box 1 motif in the 42301-42480 site. The 3′ side exon adjacent to the exon containing the Trp-Ser-Xaa-Trp-Ser motif has a transmembrane domain, and this domain has a low homology with other hemopoietin receptors, and was not detected by the BlastX scan. These results suggested the possibility of a novel hemopoietin receptor gene existing in the above-described BAC clone CIT987-SKA-670B5.

[0162]

[Example 2] Search for NR8 expressing tissues using RT-PCR

Pseudogenes have been reported to exist in several hemopoietin receptors (Kermouni, A. et al., Genomics, 1995, 29 (2) 371-382; Fukunaga,

R. and Nagata, S., Eur. J. Biochem., 1994, 220, 881-891). To verify that NR8 is not a pseudogene, and with the objective of identifying NR8 expressing tissues, transcripts of the NR8 gene were searched by RT-PCR method.

[0163]

In the AC002303 sequence of the above-described BAC clone, several exon regions widely conserved at the amino acid translation level in known cytokine receptors were surmised, and on the sequence of the surmised exon region, the following primers were synthesized. (See Fig. 5 for the location of each primer.)

NR8-SN1; 5′- CCG GCT CCC CCT TTC AAC GTG ACT GTG ACC -3′ (SEQ ID NO: 9)

NR8-SN2; 5′- GGC AAG CTT CAG TAT GAG CTG CAG TAC AGG -3′ (SEQ ID NO: 10)

NR8-AS1; 5′- ACC CTC TGA CTG GGT CTG AAA GAT GAC CGG -3′ (SEQ ID NO: 11)

NR8-AS2; 5′- CAT GGG CCC TGC CCG CAC CTG CAG CTC ATA -3′ (SEQ ID NO: 12)

Using the Human Fetal Multiple Tissue cDNA Panel (Clontech #K1425-1) as the template, RT-PCR was attempted using combinations of the above primers. Advantage cDNA Polymerase Mix (Clontech #8417-1) was used for the PCR, which was conducted under the conditions below using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler.

[0164]

Namely, the PCR conditions were, 94℃ for 4 min, 5 cycles of "94℃ for 20 sec, 72℃ for 3 min," 5 cycles of "94℃ for 20 sec, 70℃ for 3 min," 28 cycles of "94℃ for 20 sec, 68℃ for 3 min," 72℃ for 4 min, and completed at 4℃.

[0165]

From the primer locations shown in Fig. 5, amplifications of bands sized 330 bp, 258 bp, 234 bp, and 162 bp can be expected from the combinations of SN1/AS1, SN1/AS2, SN2/AS1, and SN2/AS2. When evaluated using human fetal liver, brain, and skeletal muscle cDNA as the template, clear bands having the anticipated sizes were obtained in the fetal liver only with the respective primer combinations (Fig. 3).

[0166]

An amplification was not seen at all for fetal brain cDNA, and a band of about 650 bp and a broad band of 400 to 500 bp were observed for fetal skeletal muscle cDNA. However, since the band sizes for skeletal muscle cDNA remained constant even when different combinations of primers were used, it is thought that these bands were non-specific amplifications due to some reason.

[0167]

The obtained PCR product was subcloned to pGEM-T Easy vector (Promega #A1360), and the nucleotide sequence was determined. The recombination of PCR products to the pGEM-T Easy vector was done by T4 DNA Ligase (Promega #A1360) reacted at 4℃ for 12 hr. The genetic recombinant between the PCR product and pGEM-T Easy vector was obtained by transforming *E.coli* strain DH5α (Toyobo #DNA-903).

[0168]

For the selection of the genetic recombinant, Insert Check Ready (Toyobo #PIK-101) was used. The dRhodamine Terminator Cycle Sequencing Kit (ABI/Perkin Elmer #4303141) was used for determining the nucleotide sequence, and analysis was done using the ABI PRISM 377 DNA Sequencer. As a result of determining the nucleotide sequences of all inserts of the 10 independent clones of genetic recombinants, all clones were found to comprise a single nucleotide sequence. These obtained sequences were verified to be partial nucleotide sequences of NR8.

[0169]

[Example 3] Full-length cDNA cloning by the 5′ and 3′-RACE methods

Using the thus-obtained fetal liver-derived cDNA, 5′ and 3′-RACE methods were conducted to obtain full-length cDNA (Fig. 4).

[0170]

3-1) 5′-RACE method

5′-RACE PCR was performed using the above-mentioned NR8-AS1 primer for primary PCR, and NR8-AS2 primer for secondary PCR. Human Fetal Liver Marathon-Ready cDNA Library (Clontech #7403-1) was used as the template and Advantage cDNA Polymerase Mix for the PCR experiment. As a result of PCR under the following conditions using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, two types of PCR products

were obtained, which have different sizes through selective splicing.

[0171]

Primary PCR conditions were 94℃ for 4 min, 5 cycles of "94℃ for 20 sec, 72℃ for 4 min," 5 cycles of "94℃ for 20 sec, 70℃ for 4 min," 28 cycles of "94℃ for 20 sec, 68℃ for 4 min," 72℃ for 4 min, and completed at 4℃.

[0172]

Secondary PCR conditions were 94℃ for 4 min, 5 cycles of "94℃ for 20 sec, 70℃ for 3 min 30 sec," 28 cycles of "94℃ for 20 sec, 68℃ for 3 min 30 sec," 72℃ for 4 min, and completed at 4℃.

[0173]

Both types of PCR products obtained were subcloned to pGEM-T Easy vector as mentioned earlier, and the nucleotide sequences of all inserts were determined for the 16 independent clones of genetic transformants. As before, the dRhodamine Terminator Cycle Sequencing Kit was used for determining the nucleotide sequence, and analysis was done using the ABI PRISM 377 DNA Sequencer. As a result, the clones can be divided into two groups, one having 14 clones, and the other having 2 clones, by the length of the base pairs and the differences in sequence (though described later, the differences lie in the products due to selective splicing, and the group of 14 independent clones comprises the sequence corresponding to exon 5 in the genomic sequence, and the remaining group of two independent clones does not have this sequence).

[0174]

3-2) 3'-RACE method

3'-RACE PCR was performed using the above-mentioned NR8-SN1 primer for primary PCR, and NR8-SN2 primer for secondary PCR. Human Fetal Liver Marathon-Ready cDNA Library was used as the template similar to 5'-RACE PCR, and Advantage cDNA Polymerase Mix for the PCR experiment. As a result of conducting PCR under the conditions shown in 3-1), a single band PCR product was obtained.

[0175]

The obtained PCR product was subcloned to pGEM-T Easy vector as above, and the nucleotide sequences of all inserts of the 12 independent clones of genetic recombinants were determined. As before, the dRhodamine Terminator Cycle Sequencing Kit was used for determining

the nucleotide sequence, and the sequences determined were analyzed using the ABI PRISM 377 DNA Sequencer. As a result, all 12 independent clones showed a single nucleotide sequence.

[0176]

As a result of analyzing the nucleotide sequence of the fragments (approximately 1.1 kb and 1.2 kb) amplified by 5′-RACE and 3′-RACE, respectively, it was conceived that the approximately 260 bp of each fragment overlap and extend to the 5′ side and 3′ side, and contain almost the full-length of NR8 mRNA. These were joined to make a full-length cDNA (NR8α) (Fig. 5 and Fig. 6). The plasmid containing the NR8α cDNA (SEQ ID NO: 2) was named pGEM-NR8α, and *E. coli* containing the plasmid has been internationally deposited at the National Institute of BioScience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan 305-8566) under the accession number FERM BP-6543 since October 9, 1998 according to the Budapest Treaty.

[0177]

As shown in Fig. 5 and Fig. 6, in the ORF of NR8α cDNA, the Met starting from nucleotide no. 441 is thought to be the start codon due to the presence of an inframe stop codon 39 bp upstream, and completes with two stop codons starting from nucleotide no. 1524. It has the features of, from the N terminus in order, a typical secretion signal sequence, a domain thought to be the ligand binding site containing a Cys residue conserved in other hemopoietic receptor members, a Pro-rich motif, Trp-Ser-Xaa-Trp-Ser motif, a transmembrane domain, a Box 1 motif thought to be involved in signal transduction, and such features of hemopoietin receptors. From the above results, the NR8 gene was thought to encode a novel hemopoietin receptor.

[0178]

Analysis of fragments amplified by the RACE method suggested the presence of a splice variant. As a result of nucleotide sequence analysis, this variant was revealed to be lacking approximately 150 bp including the above-described Pro-rich motif of NR8α. Moreover, as a result of comparing AC002303 sequence with NR8α, and carrying out analogy of exons/introns (Table 9), the above-described variant was thought to be deficient of the 5^{th} exon due to selective splicing.

[0179]

[Table 9]

Exon	# in AC002303	# in NR8	Characteristics
1	<1	: 1-424	inframe stop codon
2	26334-26398	: 425-489	start codon, signal peptide
3	30625-30727	: 490-592	conserved Cys residue
4	33766-33965	: 593-792	conserved Cys residue, N-glycosylation site
5	39240-39394	: 793-947	Pro-rich motif (PAPPF), N-glycosylation site
6	40820-40997	: 948-1125	gtWSEWSdp motif
7	41455-41554	: 1126-1225	transmembrane domain
8	42285-42366	: 1226-1307	Box1 (IWAVPSP)
9a	44812-44909	: 1308-1405*	connects to exon 10, Box2-like sequence (PSTLEVYSCH), nontypical exon/intron boundary
9b	44812-45922<	: 1308-2465**	double stop codons, Box2-like sequence (PSTLEVYSCH, PAELVESDG), polyA
10	45441-45922<	: 1406-1934*	double stop codons, polyA

NR8 α*: exons 1+2+3+4+5+6+7+8+9a+10

NR8 β: exons 1+2+3+4+6+7+8+9a+10

(two alternative reading frames for soluble-type and transmembrane(-signal)-type)

NR8 γ**: exons 1+2+3+4+5+6+7+8+9b

[0180]

This variant (NR8β) can encode a soluble receptor in the truncated form by the joining of the 6th exon directly to the 4th exon and causing a frame shift. The boundary between the exons and the introns takes a consensus sequence in most cases, but the boundary between the 9th exon (Exon 9a) and the 9th intron is the only boundary that takes a different sequence from the consensus sequence (nag/gtgagt, etc.), being acc/acggag. The plasmid comprising NR8β cDNA (SEQ ID NO: 2) was named pGEM-NR8β, and *E.coli* comprising the plasmid has been internationally deposited at the National Institute of BioScience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the accession number FERM BP-6544 since October 9, 1998 according to the Budapest Treaty.

[0181]

[Example 4] Northern blotting

In order to analyze the distribution and mode of NR8 gene expression in each human organ and human cancer cell lines, Northern blot analysis was done using the cDNA encoding the full-length NR8α protein prepared based on all the cDNA fragments obtained in Example 3 as a probe. The probe was prepared using Mega Prime Kit (Amersham, cat#RPN1607) and radiolabeled with [α-^{32}P] dCTP (Amersham, cat#AA0005).

[0182]

As Northern blots, Human Multiple Tissue Northern (MTN) Blot (Clontech #7760-1), Human MTN Blot IV (Clontech #7766-1), and Human Cancer Cell Line MTN Blot (Clontech #7757-1) were used. Express Hyb Hybridization Solution (Clontech #8015-2) was used for hybridization.

[0183]

Hybridization conditions were: a prehybridization at 68℃ for 30 min, followed by hybridization at 68℃ for 14 hr. After washing under the following conditions, the blots were exposed to Imaging Plate (FUJI#BAS-III), and the gene expression of NR8 mRNA was detected by the Image Analyzer (FUJIX, BAS-2000 II). Washing conditions were: (1) 1x SSC/0.1% SDS, at room temperature for 5 min; (2) 1x SSC/0.1% SDS, at 50℃ 30 min; and (3) 0.1x SSC/0.1% SDS, at 50℃ 30 min.

[0184]

Fig. 12 shows the results of Northern blot analysis of NR8 expression

in each organ. A total of three different-sized mRNA, one 5kb-sized and two 3 to 4kb sized, were detected in human adult lung, spleen, thymus, skeletal muscle, pancreas, small intestines, peripheral leucocytes, and uterus. A similar examination of various cell lines including hemopoietic cell lines showed similar sized bands in two cell lines, the promyeloid leukemic cell line HL60 and Burkett's lymphoma-derived Raji.

[0185]

[Example 5] Plaque screening

Northern blot analysis of NR8 gene expression detected at least three types of specific mRNA bands with different sizes in each human organ and in each human cancer cell line for which NR8 gene expression was seen. However, the inventors had succeeded in isolating only two types of selective splicing variants, namely NR8α and NR8β genes, in the above-described Examples. Therefore, the inventors performed plaque screening with the objective of isolating the gene of the third selective splicing variant. Human Lymph Node (Clontech, cat#HL5000a) that showed a strong NR8 gene expression in the above-mentioned Northern analysis results, was used as the cDNA library. The probe used was NR8α cDNA fragment, which was radio-labeled by [α-^{32}P] dCTP (Amersham, cat#AA0005) using the Mega Prime Kit (Amersham, cat#RPN1607). Approximately 7.2 x 10^5 plagues of Human Lymph Node cDNA Library were blotted onto a Hybond N (+) (Amersham, cat#RPN303B) charged nylon membrane to conduct primary screening. Rapid Hybridization Buffer (Amersham, cat#RPN1636) was used for the hybridization. Hybridization conditions were: a prehybridization at 65℃ for 1 hr, followed by hybridization at 65℃ for 14 hr. After washing under the conditions, (1) 1x SSC/0.1% SDS, at room temperature for 15 min; (2) 1x SSC/0.1% SDS, at 58℃ 30 min; and (3) 0.1x SSC/0.1% SDS, at 58℃ 30 min, the membrane was exposed to an X-ray film (Kodak, cat#165-1512) to detect NR8 positive plaques.

[0186]

As a result, positive or pseudo-positive 16 independent clones were obtained. A similar secondary screening was done for the 16 clones obtained from the primary screening to successfully isolate plaques of NR8 positive 15 independent clones. The inserts of these 15 clones

were amplified by PCR through a pair of primers located in both ends of the λgt10 vector cloning site. Advantage cDNA polymerase Mix (Clontech #8417-1) was used for the PCR reaction conducted using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, under the following experiment conditions. Namely, 94℃ for 4 min, 5 cycles of "94℃ for 20 sec, 70℃ for 4 min," 30 cycles of "94℃ for 20 sec, 68℃ for 4 min," 72℃ for 4 min, and completed at 4℃.

[0187]

Similar to above, the obtained PCR products were subcloned to pGEM-T Easy vector, and the nucleotide sequence of the inserts were determined using the BigDye Terminator Cycle Sequencing SF Ready Reaction Kit (ABI/Perkin Elmer#4303150), and analyzed by the ABI PRISM 377 DNA Sequencer. As a result, among the 15 clones obtained, at least two clones showed an insertion of 177 amino acids flanking the NR8α C terminus, and since this portion derives from the 9th intron of the NR8 gene and is removed by splicing in NR8α, this 3rd selective splicing variant was named NR8γ. The plasmid containing the NR8γ cDNA (SEQ ID NO: 2) was named pGEM-NR8γ, and *E.coli* containing the plasmid has been internationally deposited at the National Institute of BioScience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the accession number FERM BP-6545 since October 9, 1998 according to the Budapest Treaty.

[0188]

[Example 6] Ligand screening

6-1) Construction of NR8 chimeric receptor

A screening system was constructed for searching a ligand that can specifically bind to NR8, namely, a novel hemopoietin. First, the cDNA sequence encoding the extracellular region of NR8α (the amino acid sequence of SEQ ID NO: 1; from the 1st Met to the 228th Glu) was amplified by PCR, and this DNA fragment was bound to DNA fragments encoding the transmembrane region and the intracellular region of a known hemopoietin receptor to prepare a fusion sequence encoding a chimeric receptor. As described above, there were several candidates for the partner, the known hemopoietin receptor, and among them, the human TPO receptor (Human MPL-P) was selected. Namely, after

amplifying the DNA sequence encoding the intracellular region that includes the transmembrane region of the human TPO receptor by PCR, this sequence was bound to the cDNA sequence encoding the extracellular region of NR8α in frame, and inserted into a plasmid vector expressible in mammalian cells. The expression vector constructed was named pEF-NR8/TPO-R. A schematic diagram of the structure of the constructed NR8/TPO-R chimeric receptor is shown in Fig. 14, and the nucleotide sequence of the chimeric receptor and the expressible amino acid sequence encoded by it are shown in SEQ ID NOs: 13 and 14, respectively. Together with an expression vector pSV2bsr (Kaken Pharmaceutical Co., Ltd.) containing Blastcidin S resistant gene, the NR8/TPO-R chimeric receptor-expressing vector was introduced into the growth factor-dependent cell line Ba/F3, and forced expression was done.. Gene-introduced cells were selected by culturing with 8 μg/ml of Blastcidin S hydrochloride (Kaken Pharmaceutical Co., Ltd.) and IL-3. By transferring the obtained chimeric receptor-introduced cells to an IL-3-free medium, adding a material expected to contain a target ligand, and culturing, it is possible to conduct a screening that uses the fact that survival/proliferation will be possible only when a ligand that specifically binds to NR8 is present.

[0189]

6-2) Preparation of NR8/IgG1-Fc soluble fusion protein

NR8/IgG1-Fc soluble fusion protein was prepared to be used for searching cell membrane-bound ligands, or the detection of soluble ligands through BIAcore (Pharmacia) and West-western blotting. A fusion sequence encoding the soluble fusion protein was prepared by binding a DNA fragment encoding the extracellular region of NR8α (amino acid sequence; from the 1st Met to the 228th Glu) prepared in 5-1) with the DNA fragment encoding the Fc region of human immunoglobulin IgG1 in frame. A schematic diagram of the structure of the soluble fusion protein encoding the NR8/IgG1-Fc is shown in Fig. 14, and the nucleotide sequence and the expressible amino acid sequence encoded by it in SEQ ID NOs: 15 and 16, respectively. This fusion gene fragment was inserted into a plasmid vector expressible in mammalian cells, and the constructed expression vector was named pEF-NR8/IgG1-Fc. After forced expression of this pEF-NR8/IgG1-Fc in mammalian cells, and

selection of stable gene-introduced cells, the recombinant protein secreted into the culture supernatant can be purified by immunoprecipitation using anti-human IgG1-Fc antibody, or by affinity columns, etc.

[0190]

6-3) Construction of an expression system of NR8β and purification of recombinant NR8β protein

The recombinant NR8β protein was prepared to be used for searching cell membrane-bound ligands, or the detection of soluble ligands using BIAcore (Pharmacia) or West-western-blotting. Using the amino acid coding sequence of NR8β cDNA, the stop codon was replaced by point mutation to a nucleotide sequence encoding an arbitrary amino acid residue, and then, was bound to the nucleotide sequence encoding the FLAG peptide in frame. This bound fragment was inserted into a plasmid vector expressible within mammalian cells, and the constructed expression vector was named pEF-BOS/NR8β FLAG. Fig. 14 shows a schematic diagram of the structure of the insert NR8β FLAG within the constructed expression vector. Moreover, the nucleotide sequence of NR8β FLAG and the expressible amino acid sequence encoded by it are shown in SEQ ID NOs: 17 and 18, respectively. After forced-expression of this pEF-BOS/NR8β FLAG in mammalian cells and selection of stable gene-introduced cells, the recombinant protein secreted into the culture supernatant can be immunoprecipitated using anti-FLAG peptide antibody, or may be purified by affinity columns, etc.

[0191]

[Effects of the Invention]

The present invention provides a novel hemopoietin receptor protein, and the encoding DNA. The present invention also provides, a vector into which the DNA has been inserted, a transformant harboring the DNA, and a method of producing a recombinant protein using the transformant. It also provides a method of screening a compound or a natural ligand that binds to the protein. The protein of the invention is thought to be related to hemopoiesis, and therefore, is useful in experiments for analyzing hemopoietic functions. The protein could also be applied in the diagnosis and treatment of hemopoiesis-associated diseases.

[Sequence Listing]

SEQUENCE LISTING

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<151> 1998-6-24

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30 35 40

Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys
45 50 55

Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr

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His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp
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Phe Asn Val Thr Val Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg
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Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn
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Pro His Leu Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala
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Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile

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                160                 165                 170

agg aga aag ctg atc tca gtg gac tca aga agt gtc tcc ctc ctc ccc     1001
Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro
            175                 180                 185

ctg gag ttc cgc aaa gac tcg agc tat gag ctg cag gtg cgg gca ggg     1049
Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly
        190                 195                 200

ccc atg cct ggc tcc tcc tac cag ggg acc tgg agt gaa tgg agt gac     1097
Pro Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp
    205                 210                 215

ccg gtc atc ttt cag acc cag tca gag gag tta aag gaa ggc tgg aac     1145
Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn
220                 225                 230                 235

cct cac ctg ctg ctt ctc ctc ctg ctt gtc ata gtc ttc att cct gcc     1193
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ttc tgg agc ctg aag acc cat cca ttg tgg agg cta tgg aag aag ata     1241
Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile
            255                 260                 265

tgg gcc gtc ccc agc cct gag cgg ttc ttc atg ccc ctg tac aag ggc     1289
Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly
        270                 275                 280

tgc agc gga gac ttc aag aaa tgg gtg ggt gca ccc ttc act ggc tcc     1337
Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser
    285                 290                 295

agc ctg gag ctg gga ccc tgg agc cca gag gtg ccc tcc acc ctg gag     1385
Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu
300                 305                 310                 315

gtg tac agc tgc cac cca ccc agc agc cct gtg gag tgt gac ttc acc     1433
Val Tyr Ser Cys His Pro Pro Ser Ser Pro Val Glu Cys Asp Phe Thr
                320                 325                 330

agc ccc ggg gac gaa gga ccc ccc cgg agc tac ctc cgc cag tgg gtg     1481
Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp Val
            335                 340                 345

gtc att cct ccg cca ctt tcg agc cct gga ccc cag gcc agc taa         1526
Val Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
        350                 355                 360

tgaggctgac tggatgtcca gagctggcca ggccactggg ccctgagcca gagacaaggt   1586

cacctgggct gtgatgtgaa gacacctgca gcctttggtc tcctggatgg gcctttgagc   1646

ctgatgttta cagtgtctgt gtgtgtgtgc atatgtgtgt gtgtgcatat gcatgtgtgt   1706

gtgtgtgtgt gtcttaggtg cgcagtggca tgtccacgtg tgtgtgattg cacgtgcctg   1766
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tgggcctggg ataatgccca tggtactcca tgcattcacc tgccctgtgc atgtctggac 1826

tcacggagct cacccatgtg cacaagtgtg cacagtaaac gtgtttgtgg tcaacaga 1884

<210> 3
<211> 144
<212> PRT
<213> Homo sapiens

<400> 3

Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu
1 5 10

Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr
15 20 25

Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His
30 35 40

Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys
45 50 55

Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr
60 65 70 75

His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp
80 85 90

Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln
95 100 105

Glu Cys Gly Ser Phe Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala
110 115 120

Asp Leu Ser Gly Leu Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro
125 130 135

Gln Arg Leu Glu Leu
140

<210> 4
<211> 1729
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (441)..(872)

<400> 4
ggcagccagc ggcctcagac agacccactg gcgtctctct gctgagtgac cgtaagctcg 60

gcgtctggcc ctctgcctgc ctctccctga gtgtggctga cagccacgca gctgtgtctg 120

tctgtctgcg gcccgtgcat ccctgctgcg gccgcctggt accttccttg ccgtctcttt 180

cctctgtctg ctgctctgtg ggacacctgc ctggaggccc agctgcccgt catcagagtg 240

acaggtctta tgacagcctg attggtgact cgggctgggt gtggattctc accccaggcc 300

tctgcctgct ttctcagacc ctcatctgtc acccccacgc tgaacccagc tgccaccccc 360

agaagcccat cagactgccc ccagcacacg gaatggattt ctgagaaaga agccgaaaca 420

gaaggcccgt gggagtcagc atg ccg cgt ggc tgg gcc gcc ccc ttg ctc ctg 473
Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu
1 5 10

ctg ctg ctc cag gga ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc 521
Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr
15 20 25

```
gat tac ctc cag acg gtc atc tgc atc ctg gaa atg tgg aac ctc cac      569
Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His
         30                  35                  40

ccc agc acg ctc acc ctt acc tgg caa gac cag tat gaa gag ctg aag      617
Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys
     45                  50                  55

GAC GAG GCC ACC TCC TGC AGC CTC CAC AGG TCG GCC CAC AAT GCC ACG      665
Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr
 60                  65                  70                  75

CAT GCC ACC TAC ACC TGC CAC ATG GAT GTA TTC CAC TTC ATG GCC GAC      713
His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp
                 80                  85                  90

GAC ATT TTC AGT GTC AAC ATC ACA GAC CAG TCT GGC AAC TAC TCC CAG      761
Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln
             95                  100                 105

GAG TGT GGC AGC TTT CTC CTG GCT GAG AGC AAG TCC GAG GAG AAA GCT      809
Glu Cys Gly Ser Phe Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala
        110                 115                 120

gat ctc agt gga ctc aag aag tgt ctc cct cct ccc cct gga gtt ccg      857
Asp Leu Ser Gly Leu Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro
    125                 130                 135

caa aga ctc gag cta tgagctgcag gtgcgggcag ggcccatgcc tggctcctcc      912
Gln Arg Leu Glu Leu
140

taccagggga cctggagtga atggagtgac ccggtcatct ttcagaccca gtcagaggag    972

ttaaaggaag gctggaaccc tcacctgctg cttctcctcc tgcttgtcat agtcttcatt   1032
```

cctgccttct ggagcctgaa gacccatcca ttgtggaggc tatggaagaa gatatgggcc 1092

gtccccagcc ctgagcggtt cttcatgccc ctgtacaagg gctgcagcgg agacttcaag 1152

aaatgggtgg gtgcaccctt cactggctcc agcctggagc tgggaccctg gagcccagag 1212

gtgccctcca ccctggaggt gtacagctgc cacccaccca gcagccctgt ggagtgtgac 1272

ttcaccagcc ccggggacga aggacccccc cggagctacc tccgccagtg ggtggtcatt 1332

cctccgccac tttcgagccc tggaccccag gccagctaat gaggctgact ggatgtccag 1392

agctggccag gccactgggc cctgagccag agacaaggtc acctgggctg tgatgtgaag 1452

acacctgcag cctttggtct cctggatggg cctttgagcc tgatgtttac agtgtctgtg 1512

tgtgtgtgca tatgtgtgtg tgtgcatatg catgtgtgtg tgtgtgtgtg tcttaggtgc 1572

gcagtggcat gtccacgtgt gtgtgattgc acgtgcctgt gggcctggga taatgcccat 1632

ggtactccat gcattcacct gccctgtgca tgtctggact cacggagctc acccatgtgc 1692

acaagtgtgc acagtaaacg tgtttgtggt caacaga 1729

<210> 5
<211> 237
<212> PRT
<213> Homo sapiens

<400> 5

Met	Pro	Arg	Met	Pro	Pro	Thr	Pro	Ala	Thr	Trp	Met	Tyr	Ser	Thr	Ser
1				5				10					15		

Trp	Pro	Thr	Thr	Phe	Ser	Val	Ser	Thr	Ser	Gln	Thr	Ser	Leu	Ala	Thr
		20					25					30			

Thr Pro Arg Ser Val Ala Ala Phe Ser Trp Leu Arg Ala Ser Pro Arg
35 40 45

Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu
50 55 60

Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro
65 70 75 80

Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro
85 90 95

Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn Pro
100 105 110

His Leu Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala Phe
115 120 125

Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile Trp
130 135 140

Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly Cys
145 150 155 160

Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser Ser
165 170 175

Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu Val
180 185 190

Tyr Ser Cys His Pro Pro Ser Ser Pro Val Glu Cys Asp Phe Thr Ser
195 200 205

Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp Val Val
210 215 220

Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
225 230 235

<210> 6
<211> 1729
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (659)..(1368)

<400> 6
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gcgtctggcc ctctgcctgc ctctccctga gtgtggctga cagccacgca gctgtgtctg 120

tctgtctgcg gcccgtgcat ccctgctgcg gccgcctggt accttccttg ccgtctcttt 180

cctctgtctg ctgctctgtg ggacacctgc ctggaggccc agctgcccgt catcagagtg 240

acaggtctta tgacagcctg attggtgact cgggctgggt gtggattctc accccaggcc 300

tctgcctgct ttctcagacc ctcatctgtc acccccacgc tgaacccagc tgccaccccc 360

agaagcccat cagactgccc ccagcacacg gaatggattt ctgagaaaga agccgaaaca 420

gaaggcccgt gggagtcagc atgccgcgtg gctgggccgc ccccttgctc ctgctgctgc 480

tccagggagg ctggggctgc cccgacctcg tctgctacac cgattacctc cagacggtca 540

tctgcatcct ggaaatgtgg aacctccacc ccagcacgct cacccttacc tggcaagacc 600

agtatgaaga gctgaaggac gaggccacct cctgcagcct ccacaggtcg gcccacaa 658

atg cca cgc atg cca cct aca cct gcc aca tgg atg tat tcc act tca 705

```
Met Pro Arg Met Pro Pro Thr Pro Ala Thr Trp Met Tyr Ser Thr Ser
  1               5                  10                  15

tgg ccg acg aca ttt tca gtg tca aca tca cag acc agt ctg gca act      753
Trp Pro Thr Thr Phe Ser Val Ser Thr Ser Gln Thr Ser Leu Ala Thr
            20                  25                  30

act ccc agg agt gtg gca gct ttc tcc tgg ctg aga gca agt ccg agg      801
Thr Pro Arg Ser Val Ala Ala Phe Ser Trp Leu Arg Ala Ser Pro Arg
        35                  40                  45

aga aag ctg atc tca gtg gac tca aga agt gtc tcc ctc ctc ccc ctg      849
Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu
    50                  55                  60

gag ttc cgc aaa gac tcg agc tat gag ctg cag gtg cgg gca ggg ccc      897
Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro
65                  70                  75                  80

atg cct ggc tcc tcc tac cag ggg acc tgg agt gaa tgg agt gac ccg      945
Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro
                85                  90                  95

gtc atc ttt cag acc cag tca gag gag tta aag gaa ggc tgg aac cct      993
Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn Pro
            100                 105                 110

cac ctg ctg ctt ctc ctc ctg ctt gtc ata gtc ttc att cct gcc ttc     1041
His Leu Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala Phe
        115                 120                 125

tgg agc ctg aag acc cat cca ttg tgg agg cta tgg aag aag ata tgg     1089
Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile Trp
    130                 135                 140

gcc gtc ccc agc cct gag cgg ttc ttc atg ccc ctg tac aag ggc tgc     1137
```

Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly Cys
145 150 155 160

agc gga gac ttc aag aaa tgg gtg ggt gca ccc ttc act ggc tcc agc 1185
Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser Ser
165 170 175

ctg gag ctg gga ccc tgg agc cca gag gtg ccc tcc acc ctg gag gtg 1233
Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu Val
180 185 190

tac agc tgc cac cca ccc agc agc cct gtg gag tgt gac ttc acc agc 1281
Tyr Ser Cys His Pro Pro Ser Ser Pro Val Glu Cys Asp Phe Thr Ser
195 200 205

ccc ggg gac gaa gga ccc ccc cgg agc tac ctc cgc cag tgg gtg gtc 1329
Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp Val Val
210 215 220

att cct ccg cca ctt tcg agc cct gga ccc cag gcc agc taatgaggct 1378
Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
225 230 235

gactggatgt ccagagctgg ccaggccact gggccctgag ccagagacaa ggtcacctgg 1438

gctgtgatgt gaagacacct gcagcctttg gtctcctgga tgggcctttg agcctgatgt 1498

ttacagtgtc tgtgtgtgtg tgcatatgtg tgtgtgtgca tatgcatgtg tgtgtgtgtg 1558

tgtgtcttag gtgcgcagtg gcatgtccac gtgtgtgtga ttgcacgtgc ctgtgggcct 1618

gggataatgc ccatggtact ccatgcattc acctgccctg tgcatgtctg gactcacgga 1678

gctcacccat gtgcacaagt gtgcacagta aacgtgtttg tggtcaacaga 1729

<210> 7

<211> 538
<212> PRT
<213> Homo sapiens

<400> 7

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                    Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu
                      1               5                  10

Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr
            15                  20                  25

Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His
        30                  35                  40

Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys
    45                  50                  55

Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr
60                  65                  70                  75

His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp
                80                  85                  90

Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln
            95                  100                 105

Glu Cys Gly Ser Phe Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro
        110                 115                 120

Phe Asn Val Thr Val Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg
    125                 130                 135

Ser Asp Tyr Glu Asp Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln
140                 145                 150                 155

Tyr Glu Leu Gln Tyr Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro
```

160 165 170

Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro
175 180 185

Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly
190 195 200

Pro Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp
205 210 215

Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn
220 225 230 235

Pro His Leu Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala
240 245 250

Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile
255 260 265

Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly
270 275 280

Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser
285 290 295

Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu
300 305 310 315

Val Tyr Ser Cys His Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu
320 325 330

Thr Glu Leu Gln Glu Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro
335 340 345

Lys Pro Ser Phe Trp Pro Thr Ala Gln Asn Ser Gly Gly Ser Ala Tyr

350 355 360

Ser Glu Glu Arg Asp Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val
365 370 375

Thr Val Leu Asp Ala Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu
380 385 390 395

Asp Asp Gly Tyr Pro Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser
400 405 410

Pro Gly Leu Glu Asp Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser
415 420 425

Cys Gly Cys Val Ser Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly
430 435 440

Ser Leu Leu Asp Arg Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp
445 450 455

Ala Gly Gly Leu Pro Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu
460 465 470 475

Ser Glu Ala Gly Ser Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp
480 485 490

Ser Gly Phe Val Gly Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe
495 500 505

Thr Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp
510 515 520

Val Val Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
525 530 535

<210> 8

<211> 2415
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (441)..(2054)

<400> 8

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gcgtctggcc ctctgcctgc ctctccctga gtgtggctga cagccacgca gctgtgtctg 120

tctgtctgcg gcccgtgcat ccctgctgcg gccgcctggt accttccttg ccgtctcttt 180

cctctgtctg ctgctctgtg ggacacctgc ctggaggccc agctgcccgt catcagagtg 240

acaggtctta tgacagcctg attggtgact cgggctgggt gtggattctc accccaggcc 300

tctgcctgct ttctcagacc ctcatctgtc acccccacgc tgaacccagc tgccaccccc 360

agaagcccat cagactgccc ccagcacacg gaatggattt ctgagaaaga agccgaaaca 420

gaaggcccgt gggagtcagc atg ccg cgt ggc tgg gcc gcc ccc ttg ctc ctg 473
Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu
1 5 10

ctg ctg ctc cag gga ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc 521
Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr
15 20 25

gat tac ctc cag acg gtc atc tgc atc ctg gaa atg tgg aac ctc cac 569
Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His
30 35 40

ccc agc acg ctc acc ctt acc tgg caa gac cag tat gaa gag ctg aag 617

```
Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys
     45                  50                  55

gac gag gcc acc tcc tgc agc ctc cac agg tcg gcc cac aat gcc acg      665
Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr
 60                  65                  70                  75

cat gcc acc tac acc tgc cac atg gat gta ttc cac ttc atg gcc gac      713
His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp
                 80                  85                  90

gac att ttc agt gtc aac atc aca gac cag tct ggc aac tac tcc cag      761
Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln
             95                  100                 105

gag tgt ggc agc ttt ctc ctg gct gag agc atc aag ccg gct ccc cct      809
Glu Cys Gly Ser Phe Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro
        110                 115                 120

ttc aac gtg act gtg acc ttc tca gga cag tat aat atc tcc tgg cgc      857
Phe Asn Val Thr Val Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg
    125                 130                 135

tca gat tac gaa gac cct gcc ttc tac atg ctg aag ggc aag ctt cag      905
Ser Asp Tyr Glu Asp Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln
140                 145                 150                 155

tat gag ctg cag tac agg aac cgg gga gac ccc tgg gct gtg agt ccg      953
Tyr Glu Leu Gln Tyr Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro
                160                 165                 170

agg aga aag ctg atc tca gtg gac tca aga agt gtc tcc ctc ctc ccc     1001
Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro
            175                 180                 185

ctg gag ttc cgc aaa gac tcg agc tat gag ctg cag gtg cgg gca ggg     1049
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```
Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly
        190                 195                 200

ccc atg cct ggc tcc tcc tac cag ggg acc tgg agt gaa tgg agt gac     1097
Pro Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp
    205                 210                 215

ccg gtc atc ttt cag acc cag tca gag gag tta aag gaa ggc tgg aac     1145
Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn
220                 225                 230                 235

cct cac ctg ctg ctt ctc ctc ctg ctt gtc ata gtc ttc att cct gcc     1193
Pro His Leu Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala
                240                 245                 250

ttc tgg agc ctg aag acc cat cca ttg tgg agg cta tgg aag aag ata     1241
Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile
            255                 260                 265

tgg gcc gtc ccc agc cct gag cgg ttc ttc atg ccc ctg tac aag ggc     1289
Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly
        270                 275                 280

tgc agc gga gac ttc aag aaa tgg gtg ggt gca ccc ttc act ggc tcc     1337
Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser
    285                 290                 295

agc ctg gag ctg gga ccc tgg agc cca gag gtg ccc tcc acc ctg gag     1385
Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu
300                 305                 310                 315

gtg tac agc tgc cac cca cca cgg agc ccg gcc aag agg ctg cag ctc     1433
Val Tyr Ser Cys His Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu
                320                 325                 330

acg gag cta caa gaa cca gca gag ctg gtg gag tct gac ggt gtg ccc     1481
```

```
Thr Glu Leu Gln Glu Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro
            335                 340                 345

aag ccc agc ttc tgg ccg aca gcc cag aac tcg ggg ggc tca gct tac    1529
Lys Pro Ser Phe Trp Pro Thr Ala Gln Asn Ser Gly Gly Ser Ala Tyr
        350                 355                 360

agt gag gag agg gat cgg cca tac ggc ctg gtg tcc att gac aca gtg    1577
Ser Glu Glu Arg Asp Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val
    365                 370                 375

act gtg cta gat gca gag ggg cca tgc acc tgg ccc tgc agc tgt gag    1625
Thr Val Leu Asp Ala Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu
380                 385                 390                 395

gat gac ggc tac cca gcc ctg gac ctg gat gct ggc ctg gag ccc agc    1673
Asp Asp Gly Tyr Pro Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser
                400                 405                 410

cca ggc cta gag gac cca ctc ttg gat gca ggg acc aca gtc ctg tcc    1721
Pro Gly Leu Glu Asp Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser
            415                 420                 425

tgt ggc tgt gtc tca gct ggc agc cct ggg cta gga ggg ccc ctg gga    1769
Cys Gly Cys Val Ser Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly
        430                 435                 440

agc ctc ctg gac aga cta aag cca ccc ctt gca gat ggg gag gac tgg    1817
Ser Leu Leu Asp Arg Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp
    445                 450                 455

gct ggg gga ctg ccc tgg ggt ggc cgg tca cct gga ggg gtc tca gag    1865
Ala Gly Gly Leu Pro Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu
460                 465                 470                 475

agt gag gcg ggc tca ccc ctg gcc ggc ctg gat atg gac acg ttt gac    1913
```

Ser Glu Ala Gly Ser Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp
480 485 490

agt ggc ttt gtg ggc tct gac tgc agc agc cct gtg gag tgt gac ttc 1961
Ser Gly Phe Val Gly Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe
495 500 505

acc agc ccc ggg gac gaa gga ccc ccc cgg agc tac ctc cgc cag tgg 2009
Thr Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp
510 515 520

gtg gtc att cct ccg cca ctt tcg agc cct gga ccc cag gcc agc taa 2057
Val Val Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
525 530 535

tgaggctgac tggatgtcca gagctggcca ggccactggg ccctgagcca gagacaaggt 2117

cacctgggct gtgatgtgaa gacacctgca gcctttggtc tcctggatgg gcctttgagc 2177

ctgatgttta cagtgtctgt gtgtgtgtgc atatgtgtgt gtgtgcatat gcatgtgtgt 2237

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tgggcctggg ataatgccca tggtactcca tgcattcacc tgccctgtgc atgtctggac 2357

tcacggagct cacccatgtg cacaagtgtg cacagtaaac gtgtttgtgg tcaacaga 2415

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  1               5                  10                  15

ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc gat tac ctc cag acg   96
Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
             20                  25                  30

gtc atc tgc atc ctg gaa atg tgg aac ctc cac ccc agc acg ctc acc   144
Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
         35                  40                  45

ctt acc tgg caa gac cag tat gaa gag ctg aag gac gag gcc acc tcc   192
Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
     50                  55                  60

tgc agc ctc cac agg tcg gcc cac aat gcc acg cat gcc acc tac acc   240
Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
 65                  70                  75                  80

tgc cac atg gat gta ttc cac ttc atg gcc gac gac att ttc agt gtc   288
Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
                 85                  90                  95

aac atc aca gac cag tct ggc aac tac tcc cag gag tgt ggc agc ttt   336
Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
            100                 105                 110
```

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ctc ctg gct gag agc atc aag ccg gct ccc cct ttc aac gtg act gtg   384
Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
        115                 120                 125

acc ttc tca gga cag tat aat atc tcc tgg cgc tca gat tac gaa gac   432
Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
    130                 135                 140

cct gcc ttc tac atg ctg aag ggc aag ctt cag tat gag ctg cag tac   480
Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
145                 150                 155                 160

agg aac cgg gga gac ccc tgg gct gtg agt ccg agg aga aag ctg atc   528
Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
                165                 170                 175

tca gtg gac tca aga agt gtc tcc ctc ctc ccc ctg gag ttc cgc aaa   576
Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
            180                 185                 190

gac tcg agc tat gag ctg cag gtg cgg gca ggg ccc atg cct ggc tcc   624
Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
        195                 200                 205

tcc tac cag ggg acc tgg agt gaa tgg agt gac ccg gtc atc ttt cag   672
Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
    210                 215                 220

acc cag tca gag acc gcc tgg atc tcc ttg gtg acc gct ctg cat cta   720
Thr Gln Ser Glu Thr Ala Trp Ile Ser Leu Val Thr Ala Leu His Leu
225                 230                 235                 240

gtg ctg ggc ctc agc gcc gtc ctg ggc ctg ctg ctg ctg agg tgg cag   768
Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu Leu Arg Trp Gln
                245                 250                 255
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ttt cct gca cac tac agg aga ctg agg cat gcc ctg tgg ccc tca ctt 816
Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu
260 265 270

cca gac ctg cac cgg gtc cta ggc cag tac ctt agg gac act gca gcc 864
Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala
275 280 285

ctg agc ccg ccc aag gcc aca gtc tca gat acc tgt gaa gaa gtg gaa 912
Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys Glu Glu Val Glu
290 295 300

ccc agc ctc ctt gaa atc ctc ccc aag tcc tca gag agg act cct ttg 960
Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Arg Thr Pro Leu
305 310 315 320

ccc ctg tgt tcc tcc cag gcc cag atg gac tac cga aga ttg cag cct 1008
Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg Arg Leu Gln Pro
325 330 335

tct tgc ctg ggg acc atg ccc ctg tct gtg tgc cca ccc atg gct gag 1056
Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu
340 345 350

tca ggg tcc tgc tgt acc acc cac att gcc aac cat tcc tac cta cca 1104
Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro
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Leu Ser Tyr Trp Gln Gln Pro
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<213> Homo sapiens

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Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
            20                  25                  30

Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
        35                  40                  45

Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
    50                  55                  60

Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65                  70                  75                  80

Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
                85                  90                  95

Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
            100                 105                 110

Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
        115                 120                 125

Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
    130                 135                 140

Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
145                 150                 155                 160

Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
                165                 170                 175
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Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
180 185 190

Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
195 200 205

Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
210 215 220

Thr Gln Ser Glu Thr Ala Trp Ile Ser Leu Val Thr Ala Leu His Leu
225 230 235 240

Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu Leu Arg Trp Gln
245 250 255

Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu
260 265 270

Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala
275 280 285

Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys Glu Glu Val Glu
290 295 300

Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Arg Thr Pro Leu
305 310 315 320

Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg Arg Leu Gln Pro
325 330 335

Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu
340 345 350

Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro
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Leu Ser Tyr Trp Gln Gln Pro
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Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
20 25 30

gtc atc tgc atc ctg gaa atg tgg aac ctc cac ccc agc acg ctc acc 144
Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 45

ctt acc tgg caa gac cag tat gaa gag ctg aag gac gag gcc acc tcc 192
Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 60

tgc agc ctc cac agg tcg gcc cac aat gcc acg cat gcc acc tac acc 240
Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 80

tgc cac atg gat gta ttc cac ttc atg gcc gac gac att ttc agt gtc 288

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Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
                 85                  90                  95

aac atc aca gac cag tct ggc aac tac tcc cag gag tgt ggc agc ttt  336
Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
            100                 105                 110

ctc ctg gct gag agc atc aag ccg gct ccc cct ttc aac gtg act gtg  384
Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
        115                 120                 125

acc ttc tca gga cag tat aat atc tcc tgg cgc tca gat tac gaa gac  432
Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
    130                 135                 140

cct gcc ttc tac atg ctg aag ggc aag ctt cag tat gag ctg cag tac  480
Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
145                 150                 155                 160

agg aac cgg gga gac ccc tgg gct gtg agt ccg agg aga aag ctg atc  528
Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
                165                 170                 175

tca gtg gac tca aga agt gtc tcc ctc ctc ccc ctg gag ttc cgc aaa  576
Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
            180                 185                 190

gac tcg agc tat gag ctg cag gtg cgg gca ggg ccc atg cct ggc tcc  624
Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
        195                 200                 205

tcc tac cag ggg acc tgg agt gaa tgg agt gac ccg gtc atc ttt cag  672
Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
    210                 215                 220

acc cag tca gag gag ccc aaa tct tgt gac aaa act cac aca tgc cca  720
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225                 230                 235                 240

ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc   768
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
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ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc   816
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
            260                 265                 270

aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc   864
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
        275                 280                 285

aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg   912
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
    290                 295                 300

cgg gag gag cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc   960
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
305                 310                 315                 320

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc   1008
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                325                 330                 335

tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc   1056
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
            340                 345                 350

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg   1104
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
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gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc   1152
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Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385                 390                 395                 400

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc   1248
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
                405                 410                 415

ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag   1296
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
            420                 425                 430

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac   1344
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
        435                 440                 445

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Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 45

Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 60

Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 80

Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
85 90 95

Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
100 105 110

Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
115 120 125

Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
130 135 140

Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
145 150 155 160

Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
165 170 175

Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
180 185 190

Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
195 200 205

Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
210 215 220

Thr Gln Ser Glu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
225 230 235 240

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
245 250 255

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
260 265 270

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
275 280 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
290 295 300

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
305 310 315 320

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
325 330 335

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
340 345 350

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
355 360 365

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
370 375 380

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385 390 395 400

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
405 410 415

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
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Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc gat tac ctc cag acg 96
Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
20 25 30

gtc atc tgc atc ctg gaa atg tgg aac ctc cac ccc agc acg ctc acc 144
Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 45

ctt acc tgg caa gac cag tat gaa gag ctg aag gac gag gcc acc tcc 192
Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 60

tgc agc ctc cac agg tcg gcc cac aat gcc acg cat gcc acc tac acc 240
Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 80

tgc cac atg gat gta ttc cac ttc atg gcc gac gac att ttc agt gtc 288
Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
85 90 95

aac atc aca gac cag tct ggc aac tac tcc cag gag tgt ggc agc ttt 336
Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
100 105 110

ctc ctg gct gag agc aag tcc gag gag aaa gct gat ctc agt gga ctc 384
Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala Asp Leu Ser Gly Leu
115 120 125

aag aag tgt ctc cct cct ccc cct gga gtt ccg caa aga ctc gag cta 432
Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro Gln Arg Leu Glu Leu
130 135 140

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20 25 30

Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 45

Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 60

Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 80

Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
85 90 95

Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
100 105 110

Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala Asp Leu Ser Gly Leu
115 120 125

Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro Gln Arg Leu Glu Leu
130 135 140

Arg Ala Arg Gln Asp Tyr Lys Asp Asp Asp Asp Lys Thr Arg
145 150 155

[Brief Description of the Drawings]

[Fig. 1]

Fig. 1 is a schematic diagram showing the results of BlastX search where the query was 180 nucleotides of 40952-40966 including 40952-40966, the only probe sequence within the AC002303. "#": For only NR8 the number was indicated by the nucleotide number. The underline of the NR8 sequence shows the portion corresponding to the exon. Other underlined sequences show identical amino acids.

[Fig. 2]

Fig. 2 is a schematic diagram showing the results of BlastX scanning of 180 nucleotides in both the 5′ and 3′ directions, where the search

centered on the 180 nucleotides of 40952-40966 containing 40952-40966, the only probe sequence within the AC002303.

[Fig. 3]

Fig. 3 shows the electrophoresis results of the amplification done by the RT-PCR method for the combinations of SN1/AS1, SN1/AS2, SN2/AS1, and SN2/AS2 primers using human fetal liver and skeletal muscle cDNA as templates.

[Fig. 4]

Fig. 4 shows the electrophoretic results of the 5′-RACE method and 3′-RACE method using human fetal liver cDNA as the template.

[Fig. 5]

Fig. 5 shows the nucleotide sequence and the amino acid sequence of NR8α cDNA. The arrows show the positions of primers used for RT-PCR. They are, SN1 (798-827), SN2 (894-923), AS2 (1055-1026), and AS1 (1127-1098) from the 5′ side, in their order. For two bases at the 5′ end of AS1, AC, which is derived from the genomic sequence, was used in place of CT.

[Fig. 6]

Fig. 6 is the continuation of Fig. 5 showing the nucleotide sequence and the amino acid sequence of NR8α cDNA.

[Fig. 7]

Fig. 7 shows the nucleotide sequence and the amino acid sequence of NR8β cDNA. Two possible open reading frames (ORF) are shown.

[Fig. 8]

Fig. 8 is the continuation of Fig. 7 showing the nucleotide sequence and the amino acid sequence of NR8β cDNA.

[Fig. 9]

Fig. 9 shows the nucleotide sequence and the amino acid sequence of NR8γ cDNA. The 177 amino acids inserted by selective splicing are underlined.

[Fig. 10]

Fig. 10 is the continuation of Fig. 9 showing the nucleotide sequence and the amino acid sequence of NR8γ cDNA. The 177 amino acids inserted by selective splicing are underlined.

[Fig. 11]

Fig. 11 is the continuation of Fig. 10 showing the nucleotide

sequence and the amino acid sequence of NR8γ cDNA.

[Fig. 12]

Fig. 12 shows the results of Northern blot analysis of NR8 expression in each organ.

[Fig. 13]

Fig. 13 is a schematic diagram showing the structure of the NR8 gene. Other repetitives include, (CA)n, (CAGA)n, (TGGA)n, (CATA)n, (TA)n, (GA)n, (GGAA)n, (CATG)n, (GAAA)n, MSTA, AT-rich, MLT1A1, LINE2, FLAM_C, MER63A, and MSTB.

[Fig. 14]

Fig. 14 is a schematic diagram showing the structure of expressible proteins constructed in the expression vector.

NR8#	40862	SLLPLEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQTQSEGRCEAGMDTPLL	41032
hTPOR	442	LELRPRSRYRLQLRAR-LNGPTYQGPWSSWSDPTRVETATE	481
hOBR	292	SLLVDSILPGSSYEVQVRGKRLDGP---GIWSDWSTPRVFTTQ	331
hIL2Rb	201	DTQYEFQVRVKPLQGEFT--TWSPWSQPLAFRTK	232
hIL7R	189	TLLQRKLQPAAMYEIKVRS--IPDHYFKGFWSEWSPSYYFRTPEINNSSGEMDPILL	243
hGM-CSFRb	196	TLGPEHLMPSSTYVARVRTRLAPGSRLSGRPSKWSPEVCWDSQ	238
	419	TGYNGIWSEWSEARSWDTES	438
mIL3Rb	200	NLEPKLFLPNSIYAARVRTRLSAGSSLSGRPSRWSPEVHWDSQ	242
	404	QLEPDTSYCARVRVKPI--SDYDGIWSEWSNEYTWTT	438
hIL5Ra	302	SKYDVQVRAAVSSMCREAGLWSEWSQPI	329
hIL9R	241	YTGQWSEWSQPVCFQ	255
hEPOR	211	RGRTRYTFAVRAR-MAEPSFGGFWSAWSEPVSLLTPSD	247
hIL2Rr	209	SLPSVDGQKRYTFRVRSRFNPLCGSAQH--WSEWSHPI	244
hIL12R	197	LCPLEMNVAQEFQLRRRQLGSQGSS----WSKWSSPV	229
hIL12Rb	282	LDLKPFTEYEFQISSKL---HLYKGSWSDWSESLRAQTPEE	319

JP Hei 10-297409

[(Query) : 39181-39360]

NR8	39233	HQVKPAPPFN--VTVTFSGQYNISWRS-DYEDP-----AFYMLKGKLQY	39355
hIL6Ra	214	LQPDPPANI--TVTAVAR-NPRWLSVTWQDPHSWNSSFYRLRFELRY	257
hgp130	218	YKVKPNPPHNL--SVINSEELSSILKLTWT-NPSIKSV--IILKYNIQY	261
rOBRb	234	VKPDPPLGLRMEVTDDGNLKISWDS-QTKAP	263

[(Query) : 42301-42480]

NR8	42307	VPSPERFFMPLYKGCSGDFK	42366
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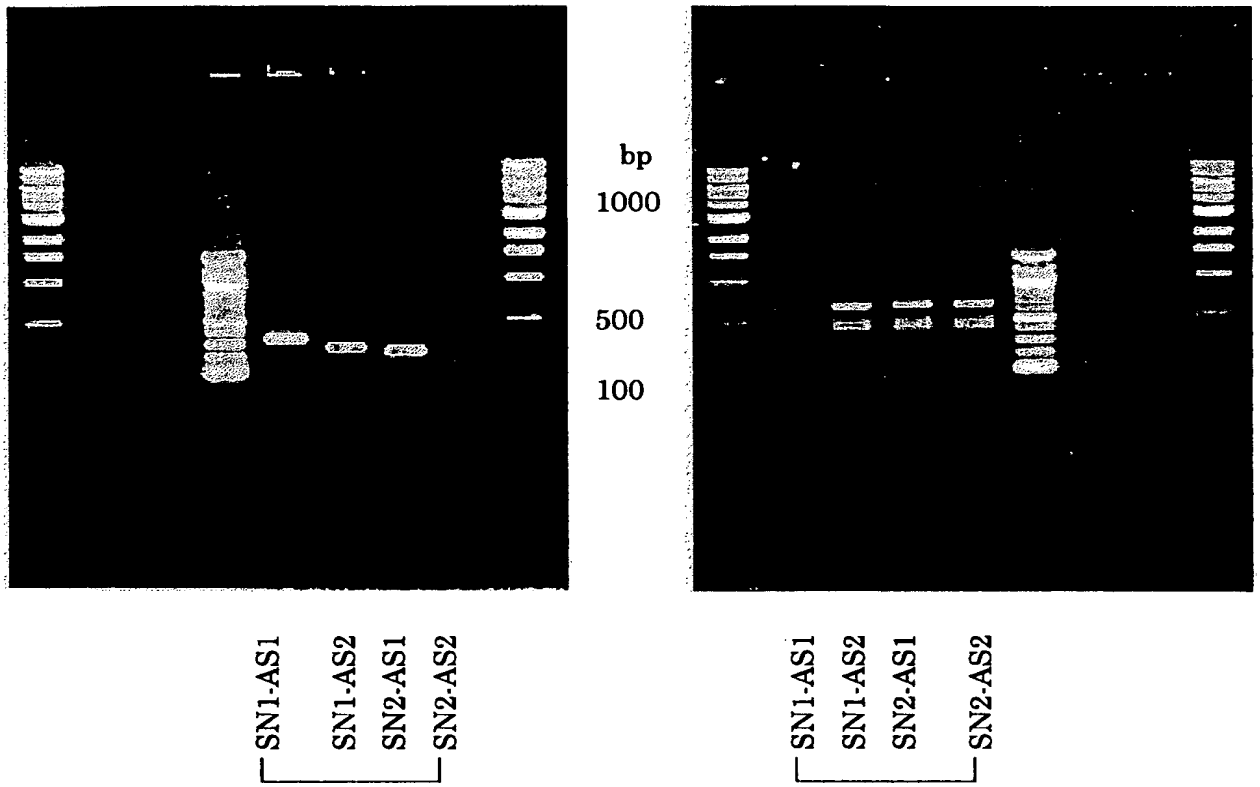
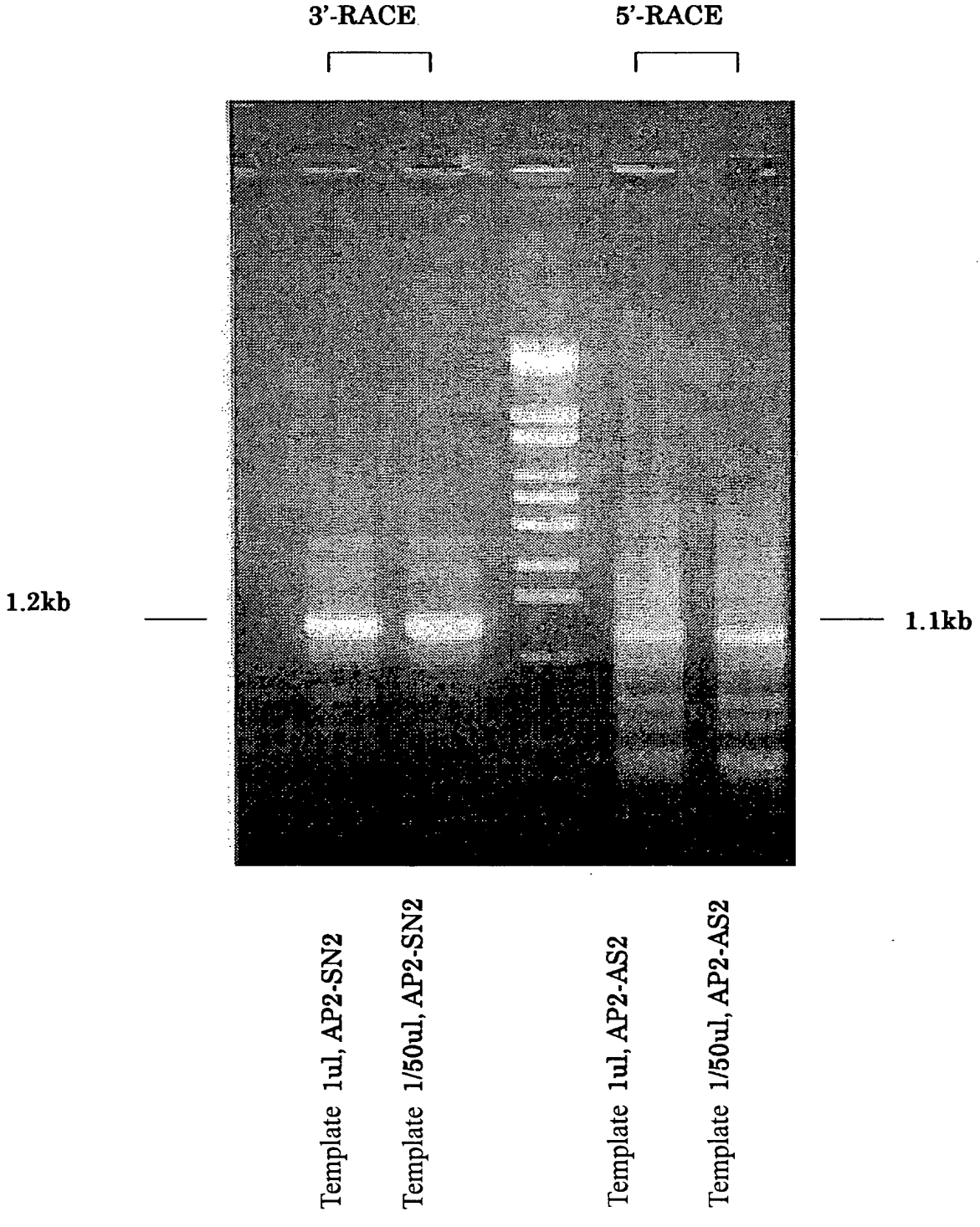
hIL9R	305	VPSPAMFFQPLYSVHNGNFQ	324

[Fig. 3]


bp
1000
500
100
SN1-AS1
SN1-AS2
SN2-AS1
SN2-AS2
Fetal liver
SN1-AS1
SN1-AS2
SN2-AS1
SN2-AS2
Fetal skeletal muscle

[Fig. 4]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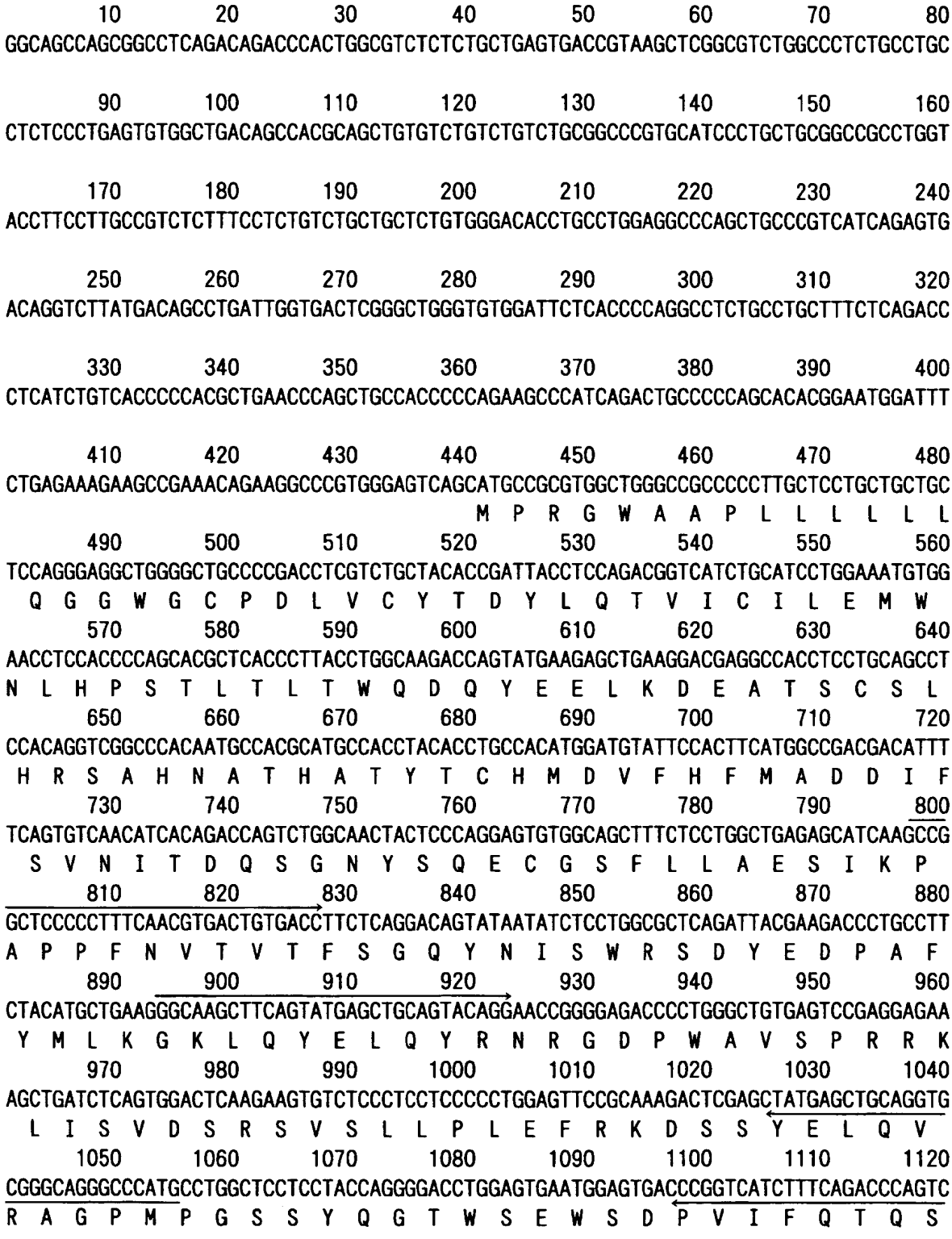
[Fig. 5]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170 180 190 200 210 220 230 240
ACCTTCCTTGCCGTCTCTTTCCTCTGTCTGCTGCTCTGTGGGACACCTGCCTGGAGGCCCAGCTGCCCGTCATCAGAGTG
250 260 270 280 290 300 310 320
ACAGGTCTTATGACAGCCTGATTGGTGACTCGGGCTGGGTGTGGATTCTCACCCCAGGCCTCTGCCTGCTTTCTCAGACC
330 340 350 360 370 380 390 400
CTCATCTGTCACCCCCACGCTGAACCCAGCTGCCACCCCCAGAAGCCCATCAGACTGCCCCCAGCACACGGAATGGATTT
410 420 430 440 450 460 470 480
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M P R G W A A P L L L L L L
490 500 510 520 530 540 550 560
TCCAGGGAGGCTGGGGCTGCCCCGACCTCGTCTGCTACACCGATTACCTCCAGACGGTCATCTGCATCCTGGAAATGTGG
Q G G W G C P D L V C Y T D Y L Q T V I C I L E M W
570 580 590 600 610 620 630 640
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N L H P S T L T L T W Q D Q Y E E L K D E A T S C S L
650 660 670 680 690 700 710 720
CCACAGGTCGGCCCACAATGCCACGCATGCCACCTACACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATTT
H R S A H N A T H A T Y T C H M D V F H F M A D D I F
730 740 750 760 770 780 790 800
TCAGTGTCAACATCACAGACCAGTCTGGCAACTACTCCCAGGAGTGTGGCAGCTTTCTCCTGGCTGAGAGCATCAAGCCG
S V N I T D Q S G N Y S Q E C G S F L L A E S I K P
810 820 830 840 850 860 870 880
GCTCCCCCTTTCAACGTGACTGTGACCTTCTCAGGACAGTATAATATCTCCTGGCGCTCAGATTACGAAGACCCTGCCTT
A P P F N V T V T F S G Q Y N I S W R S D Y E D P A F
890 900 910 920 930 940 950 960
CTACATGCTGAAGGGCAAGCTTCAGTATGAGCTGCAGTACAGGAACCGGGGAGACCCCTGGGCTGTGAGTCCGAGGAGAA
Y M L K G K L Q Y E L Q Y R N R G D P W A V S P R R K
970 980 990 1000 1010 1020 1030 1040
AGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCCTCCCCCTGGAGTTCCGCAAAGACTCGAGCTATGAGCTGCAGGTG
L I S V D S R S V S L L P L E F R K D S S Y E L Q V
1050 1060 1070 1080 1090 1100 1110 1120
CGGGCAGGGCCCATGCCTGGCTCCTCCTACCAGGGGACCTGGAGTGAATGGAGTGACCCGGTCATCTTTCAGACCCAGTC
R A G P M P G S S Y Q G T W S E W S D P V I F Q T Q S

[Fig. 6]

```
     1130       1140       1150       1160       1170       1180       1190       1200
AGAGGAGTTAAAGGAAGGCTGGAACCCTCACCTGCTGCTTCTCCTCCTGCTTGTCATAGTCTTCATTCCTGCCTTCTGGA
E  E  L  K  E  G  W  N  P  H  L  L  L  L  L  L  V  I  V  F  I  P  A  F  W  S
     1210       1220       1230       1240       1250       1260       1270       1280
GCCTGAAGACCCATCCATTGTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGCCCCTG
  L  K  T  H  P  L  W  R  L  W  K  K  I  W  A  V  P  S  P  E  R  F  F  M  P  L
     1290       1300       1310       1320       1330       1340       1350       1360
TACAAGGGCTGCAGCGGAGACTTCAAGAAATGGGTGGGTGCACCCTTCACTGGCTCCAGCCTGGAGCTGGGACCCTGGAG
Y  K  G  C  S  G  D  F  K  K  W  V  G  A  P  F  T  G  S  S  L  E  L  G  P  W  S
     1370       1380       1390       1400       1410       1420       1430       1440
CCCAGAGGTGCCCTCCACCCTGGAGGTGTACAGCTGCCACCCACCCAGCAGCCCTGTGGAGTGTGACTTCACCAGCCCCG
 P  E  V  P  S  T  L  E  V  Y  S  C  H  P  P  S  S  P  V  E  C  D  F  T  S  P  G
     1450       1460       1470       1480       1490       1500       1510       1520
GGGACGAAGGACCCCCCCGGAGCTACCTCCGCCAGTGGGTGGTCATTCCTCCGCCACTTTCGAGCCCTGGACCCCAGGCC
  D  E  G  P  P  R  S  Y  L  R  Q  W  V  V  I  P  P  P  L  S  S  P  G  P  Q  A
     1530       1540       1550       1560       1570       1580       1590       1600
AGCTAATGAGGCTGACTGGATGTCCAGAGCTGGCCAGGCCACTGGGCCCTGAGCCAGAGACAAGGTCACCTGGGCTGTGA
S  *  *
     1610       1620       1630       1640       1650       1660       1670       1680
TGTGAAGACACCTGCAGCCTTTGGTCTCCTGGATGGGCCTTTGAGCCTGATGTTTACAGTGTCTGTGTGTGTGTGCATAT

     1690       1700       1710       1720       1730       1740       1750       1760
GTGTGTGTGTGCATATGCATGTGTGTGTGTGTGTGTCTTAGGTGCGCAGTGGCATGTCCACGTGTGTGTGATTGCACG

     1770       1780       1790       1800       1810       1820       1830       1840
TGCCTGTGGGCCTGGGATAATGCCCATGGTACTCCATGCATTCACCTGCCCTGTGCATGTCTGGACTCACGGAGCTCACC

     1850       1860       1870       1880       1890       1900       1910       1920
CATGTGCACAAGTGTGCACAGTAAACGTGTTTGTGGTCAACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

     1930
AAAAAAAAAAAAA
```

[Fig. 7]

```
       10        20        30        40        50        60        70        80
GGCAGCCAGCGGCCTCAGACAGACCCACTGGCGTCTCTCTGCTGAGTGACCGTAAGCTCGGCGTCTGGCCCTCTGCCTGC

       90       100       110       120       130       140       150       160
CTCTCCCTGAGTGTGGCTGACAGCCACGCAGCTGTGTCTGTCTGTCTGCGGCCCGTGCATCCCTGCTGCGGCCGCCTGGT

      170       180       190       200       210       220       230       240
ACCTTCCTTGCCGTCTCTTTCCTCTGTCTGCTGCTCTGTGGGACACCTGCCTGGAGGCCCAGCTGCCCGTCATCAGAGTG

      250       260       270       280       290       300       310       320
ACAGGTCTTATGACAGCCTGATTGGTGACTCGGGCTGGGTGTGGATTCTCACCCCAGGCCTCTGCCTGCTTTCTCAGACC

      330       340       350       360       370       380       390       400
CTCATCTGTCACCCCCACGCTGAACCCAGCTGCCACCCCCAGAAGCCCATCAGACTGCCCCCAGCACACGGAATGGATTT

      410       420       430       440       450       460       470       480
CTGAGAAAGAAGCCGAAACAGAAGGCCCGTGGGAGTCAGCATGCCGCGTGGCTGGGCCGCCCCCTTGCTCCTGCTGCTGC
                                          M  P  R  G  W  A  A  P  L  L  L  L  L  L

      490       500       510       520       530       540       550       560
TCCAGGGAGGCTGGGGCTGCCCCGACCTCGTCTGCTACACCGATTACCTCCAGACGGTCATCTGCATCCTGGAAATGTGG
  Q  G  G  W  G  C  P  D  L  V  C  Y  T  D  Y  L  Q  T  V  I  C  I  L  E  M  W

      570       580       590       600       610       620       630       640
AACCTCCACCCCAGCACGCTCACCCTTACCTGGCAAGACCAGTATGAAGAGCTGAAGGACGAGGCCACCTCCTGCAGCCT
N  L  H  P  S  T  L  T  L  T  W  Q  D  Q  Y  E  E  L  K  D  E  A  T  S  C  S  L

      650       660       670       680       690       700       710       720
CCACAGGTCGGCCCACAATGCCACGCATGCCACCTACACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATTT
 H  R  S  A  H  N  A  T  H  A  T  Y  T  C  H  M  D  V  F  H  F  M  A  D  D  I  F
                M  P  R  M  P  P  T  P  A  T  W  M  Y  S  T  S  W  P  T  T  F
      730       740       750       760       770       780       790       800
TCAGTGTCAACATCACAGACCAGTCTGGCAACTACTCCCAGGAGTGTGGCAGCTTTCTCCTGGCTGAGAGCAAGTCCGAG
  S  V  N  I  T  D  Q  S  G  N  Y  S  Q  E  C  G  S  F  L  L  A  E  S  K  S  E
S  V  S  T  S  Q  T  S  L  A  T  T  P  R  S  V  A  A  F  S  W  L  R  A  S  P  R
      810       820       830       840       850       860       870       880
GAGAAAGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCCTCCCCCTGGAGTTCCGCAAAGACTCGAGCTATGAGCTGC
E  K  A  D  L  S  G  L  K  K  C  L  P  P  P  P  G  V  P  Q  R  L  E  L  *
 R  K  L  I  S  V  D  S  R  S  V  S  L  L  P  L  E  F  R  K  D  S  S  Y  E  L  Q
      890       900       910       920       930       940       950       960
AGGTGCGGGCAGGGCCCATGCCTGGCTCCTCCTACCAGGGGACCTGGAGTGAATGGAGTGACCCGGTCATCTTTCAGACC

  V  R  A  G  P  M  P  G  S  S  Y  Q  G  T  W  S  E  W  S  D  P  V  I  F  Q  T
```

[Fig. 8]

```
      970       980       990      1000      1010      1020      1030      1040
CAGTCAGAGGAGTTAAAGGAAGGCTGGAACCCTCACCTGCTGCTTCTCCTCCTGCTTGTCATAGTCTTCATTCCTGCCTT

Q  S  E  E  L  K  E  G  W  N  P  H  L  L  L  L  L  L  L  V  I  V  F  I  P  A  F
     1050      1060      1070      1080      1090      1100      1110      1120
CTGGAGCCTGAAGACCCATCCATTGTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGC

 W  S  L  K  T  H  P  L  W  R  L  W  K  K  I  W  A  V  P  S  P  E  R  F  F  M  P
     1130      1140      1150      1160      1170      1180      1190      1200
CCCTGTACAAGGGCTGCAGCGGAGACTTCAAGAAATGGGTGGGTGCACCCTTCACTGGCTCCAGCCTGGAGCTGGGACCC

  L  Y  K  G  C  S  G  D  F  K  K  W  V  G  A  P  F  T  G  S  S  L  E  L  G  P
     1210      1220      1230      1240      1250      1260      1270      1280
TGGAGCCCAGAGGTGCCCTCCACCCTGGAGGTGTACAGCTGCCACCCACCCAGCAGCCCTGTGGAGTGTGACTTCACCAG

W  S  P  E  V  P  S  T  L  E  V  Y  S  C  H  P  P  S  S  P  V  E  C  D  F  T  S
     1290      1300      1310      1320      1330      1340      1350      1360
CCCCGGGGACGAAGGACCCCCCCGGAGCTACCTCCGCCAGTGGGTGGTCATTCCTCCGCCACTTTCGAGCCCTGGACCCC

 P  G  D  E  G  P  P  R  S  Y  L  R  Q  W  V  V  I  P  P  P  L  S  S  P  G  P  Q

     1370      1380      1390      1400      1410      1420      1430      1440
AGGCCAGCTAATGAGGCTGACTGGATGTCCAGAGCTGGCCAGGCCACTGGGCCCTGAGCCAGAGACAAGGTCACCTGGGC

  A  S  *  *

     1450      1460      1470      1480      1490      1500      1510      1520
TGTGATGTGAAGACACCTGCAGCCTTTGGTCTCCTGGATGGGCCTTTGAGCCTGATGTTTACAGTGTCTGTGTGTGTGTG


     1530      1540      1550      1560      1570      1580      1590      1600
CATATGTGTGTGTGTGCATATGCATGTGTGTGTGTGTGTGTGTCTTAGGTGCGCAGTGGCATGTCCACGTGTGTGTGATT


     1610      1620      1630      1640      1650      1660      1670      1680
GCACGTGCCTGTGGGCCTGGGATAATGCCCATGGTACTCCATGCATTCACCTGCCCTGTGCATGTCTGGACTCACGGAGC


     1690      1700      1710      1720      1730      1740      1750      1760
TCACCCATGTGCACAAGTGTGCACAGTAAACGTGTTTGTGGTCAACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA


     1770      1780
AAAAAAAAAAAAAAAAAA
```

[Fig. 9]

```
       10        20        30        40        50        60        70        80
GGCAGCCAGCGGCCTCAGACAGACCCACTGGCGTCTCTCTGCTGAGTGACCGTAAGCTCGGCGTCTGGCCCTCTGCCTGC

       90       100       110       120       130       140       150       160
CTCTCCCTGAGTGTGGCTGACAGCCACGCAGCTGTGTCTGTCTGTCTGCGGCCCGTGCATCCCTGCTGCGGCCGCCTGGT

      170       180       190       200       210       220       230       240
ACCTTCCTTGCCGTCTCTTTCCTCTGTCTGCTGCTCTGTGGGACACCTGCCTGGAGGCCCAGCTGCCCGTCATCAGAGTG

      250       260       270       280       290       300       310       320
ACAGGTCTTATGACAGCCTGATTGGTGACTCGGGCTGGGTGTGGATTCTCACCCCAGGCCTCTGCCTGCTTTCTCAGACC

      330       340       350       360       370       380       390       400
CTCATCTGTCACCCCCACGCTGAACCCAGCTGCCACCCCCAGAAGCCCATCAGACTGCCCCCAGCACACGGAATGGATTT

      410       420       430       440       450       460       470       480
CTGAGAAAGAAGCCGAAACAGAAGGCCCGTGGGAGTCAGCATGCCGCGTGGCTGGGCCGCCCCCTTGCTCCTGCTGCTGC
                                          M  P  R  G  W  A  A  P  L  L  L  L  L  L
      490       500       510       520       530       540       550       560
TCCAGGGAGGCTGGGGCTGCCCCGACCTCGTCTGCTACACCGATTACCTCCAGACGGTCATCTGCATCCTGGAAATGTGG
  Q  G  G  W  G  C  P  D  L  V  C  Y  T  D  Y  L  Q  T  V  I  C  I  L  E  M  W
      570       580       590       600       610       620       630       640
AACCTCCACCCCAGCACGCTCACCCTTACCTGGCAAGACCAGTATGAAGAGCTGAAGGACGAGGCCACCTCCTGCAGCCT
N  L  H  P  S  T  L  T  L  T  W  Q  D  Q  Y  E  E  L  K  D  E  A  T  S  C  S  L
      650       660       670       680       690       700       710       720
CCACAGGTCGGCCCACAATGCCACGCATGCCACCTACACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATTT
 H  R  S  A  H  N  A  T  H  A  T  Y  T  C  H  M  D  V  F  H  F  M  A  D  D  I  F
      730       740       750       760       770       780       790       800
TCAGTGTCAACATCACAGACCAGTCTGGCAACTACTCCCAGGAGTGTGGCAGCTTTCTCCTGGCTGAGAGCATCAAGCCG
  S  V  N  I  T  D  Q  S  G  N  Y  S  Q  E  C  G  S  F  L  L  A  E  S  I  K  P
      810       820       830       840       850       860       870       880
GCTCCCCCTTTCAACGTGACTGTGACCTTCTCAGGACAGTATAATATCTCCTGGCGCTCAGATTACGAAGACCCTGCCTT
A  P  P  F  N  V  T  V  T  F  S  G  Q  Y  N  I  S  W  R  S  D  Y  E  D  P  A  F
      890       900       910       920       930       940       950       960
CTACATGCTGAAGGGCAAGCTTCAGTATGAGCTGCAGTACAGGAACCGGGGAGACCCCTGGGCTGTGAGTCCGAGGAGAA
 Y  M  L  K  G  K  L  Q  Y  E  L  Q  Y  R  N  R  G  D  P  W  A  V  S  P  R  R  K
      970       980       990      1000      1010      1020      1030      1040
AGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCCTCCCCCTGGAGTTCCGCAAAGACTCGAGCTATGAGCTGCAGGTG
  L  I  S  V  D  S  R  S  V  S  L  L  P  L  E  F  R  K  D  S  S  Y  E  L  Q  V
     1050      1060      1070      1080      1090      1100      1110      1120
CGGGCAGGGCCCATGCCTGGCTCCTCCTACCAGGGGACCTGGAGTGAATGGAGTGACCCGGTCATCTTTCAGACCCAGTC
R  A  G  P  M  P  G  S  S  Y  Q  G  T  W  S  E  W  S  D  P  V  I  F  Q  T  Q  S
```

[Fig. 10]

```
     1130       1140       1150       1160       1170       1180       1190       1200
AGAGGAGTTAAAGGAAGGCTGGAACCCTCACCTGCTGCTTCTCCTCCTGCTTGTCATAGTCTTCATTCCTGCCTTCTGGA
E  E  L  K  E  G  W  N  P  H  L  L  L  L  L  L  V  I  V  F  I  P  A  F  W  S
     1210       1220       1230       1240       1250       1260       1270       1280
GCCTGAAGACCCATCCATTGTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGCCCCTG
  L  K  T  H  P  L  W  R  L  W  K  K  I  W  A  V  P  S  P  E  R  F  F  M  P  L
     1290       1300       1310       1320       1330       1340       1350       1360
TACAAGGGCTGCAGCGGAGACTTCAAGAAATGGGTGGGTGCACCCTTCACTGGCTCCAGCCTGGAGCTGGGACCCTGGAG
Y  K  G  C  S  G  D  F  K  K  W  V  G  A  P  F  T  G  S  S  L  E  L  G  P  W  S
     1370       1380       1390       1400       1410       1420       1430       1440
CCCAGAGGTGCCCTCCACCCTGGAGGTGTACAGCTGCCACCCACCACGGAGCCCGGCCAAGAGGCTGCAGCTCACGGAGC
 P  E  V  P  S  T  L  E  V  Y  S  C  H  P  P  R  S  P  A  K  R  L  Q  L  T  E  L
     1450       1460       1470       1480       1490       1500       1510       1520
TACAAGAACCAGCAGAGCTGGTGGAGTCTGACGGTGTGCCCAAGCCCAGCTTCTGGCCGACAGCCCAGAACTCGGGGGGC
  Q  E  P  A  E  L  V  E  S  D  G  V  P  K  P  S  F  W  P  T  A  Q  N  S  G  G

     1530       1540       1550       1560       1570       1580       1590       1600
TCAGCTTACAGTGAGGAGAGGGATCGGCCATACGGCCTGGTGTCCATTGACACAGTGACTGTGCTAGATGCAGAGGGGCC
S  A  Y  S  E  E  R  D  R  P  Y  G  L  V  S  I  D  T  V  T  V  L  D  A  E  G  P

     1610       1620       1630       1640       1650       1660       1670       1680
ATGCACCTGGCCCTGCAGCTGTGAGGATGACGGCTACCCAGCCCTGGACCTGGATGCTGGCCTGGAGCCCAGCCCAGGCC
 C  T  W  P  C  S  C  E  D  D  G  Y  P  A  L  D  L  D  A  G  L  E  P  S  P  G  L

     1690       1700       1710       1720       1730       1740       1750       1760
TAGAGGACCCACTCTTGGATGCAGGGACCACAGTCCTGTCCTGTGGCTGTGTCTCAGCTGGCAGCCCTGGGCTAGGAGGG
  E  D  P  L  L  D  A  G  T  T  V  L  S  C  G  C  V  S  A  G  S  P  G  L  G  G

     1770       1780       1790       1800       1810       1820       1830       1840
CCCCTGGGAAGCCTCCTGGACAGACTAAAGCCACCCCTTGCAGATGGGGAGGACTGGGCTGGGGGACTGCCCTGGGGTGG
P  L  G  S  L  L  D  R  L  K  P  P  L  A  D  G  E  D  W  A  G  G  L  P  W  G  G

     1850       1860       1870       1880       1890       1900       1910       1920
CCGGTCACCTGGAGGGGTCTCAGAGAGTGAGGCGGGCTCACCCCTGGCCGGCCTGGATATGGACACGTTTGACAGTGGCT
 R  S  P  G  G  V  S  E  S  E  A  G  S  P  L  A  G  L  D  M  D  T  F  D  S  G  F

     1930       1940       1950       1960       1970       1980       1990       2000
TTGTGGGCTCTGACTGCAGCAGCCCTGTGGAGTGTGACTTCACCAGCCCCGGGGACGAAGGACCCCCCCGGAGCTACCTC
  V  G  S  D  C  S  S  P  V  E  C  D  F  T  S  P  G  D  E  G  P  P  R  S  Y  L

     2010       2020       2030       2040       2050       2060       2070       2080
CGCCAGTGGGTGGTCATTCCTCCGCCACTTTCGAGCCCTGGACCCCAGGCCAGCTAATGAGGCTGACTGGATGTCCAGAG
R  Q  W  V  V  I  P  P  P  L  S  S  P  G  P  Q  A  S  *  *
```

[Fig. 11]

```
     2090       2100       2110       2120       2130       2140       2150       2160
CTGGCCAGGCCACTGGGCCCTGAGCCAGAGACAAGGTCACCTGGGCTGTGATGTGAAGACACCTGCAGCCTTTGGTCTCC
     2170       2180       2190       2200       2210       2220       2230       2240
TGGATGGGCCTTTGAGCCTGATGTTTACAGTGTCTGTGTGTGTGTGCATATGTGTGTGTGTGCATATGCATGTGTGTGTG

     2250       2260       2270       2280       2290       2300       2310       2320
TGTGTGTGTCTTAGGTGCGCAGTGGCATGTCCACGTGTGTGTGATTGCACGTGCCTGTGGGCCTGGGATAATGCCCATGG

     2330       2340       2350       2360       2370       2380       2390       2400
TACTCCATGCATTCACCTGCCCTGTGCATGTCTGGACTCACGGAGCTCACCCATGTGCACAAGTGTGCACAGTAAACGTG

     2410       2420       2430       2440       2450       2460       2470       2480
TTTGTGGTCAACAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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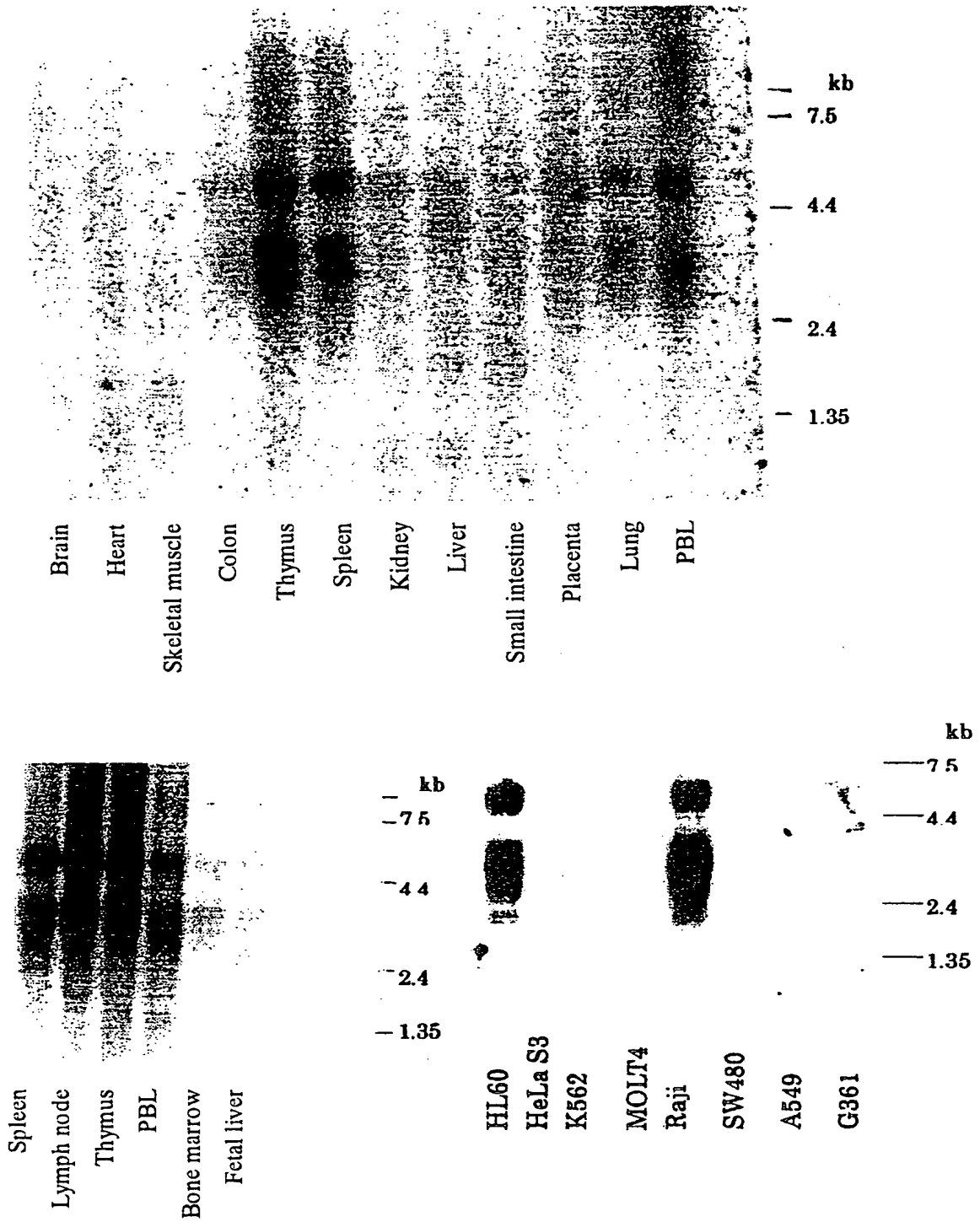
[Fig. 12]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Spleen
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PBL
Bone marrow
Fetal liver
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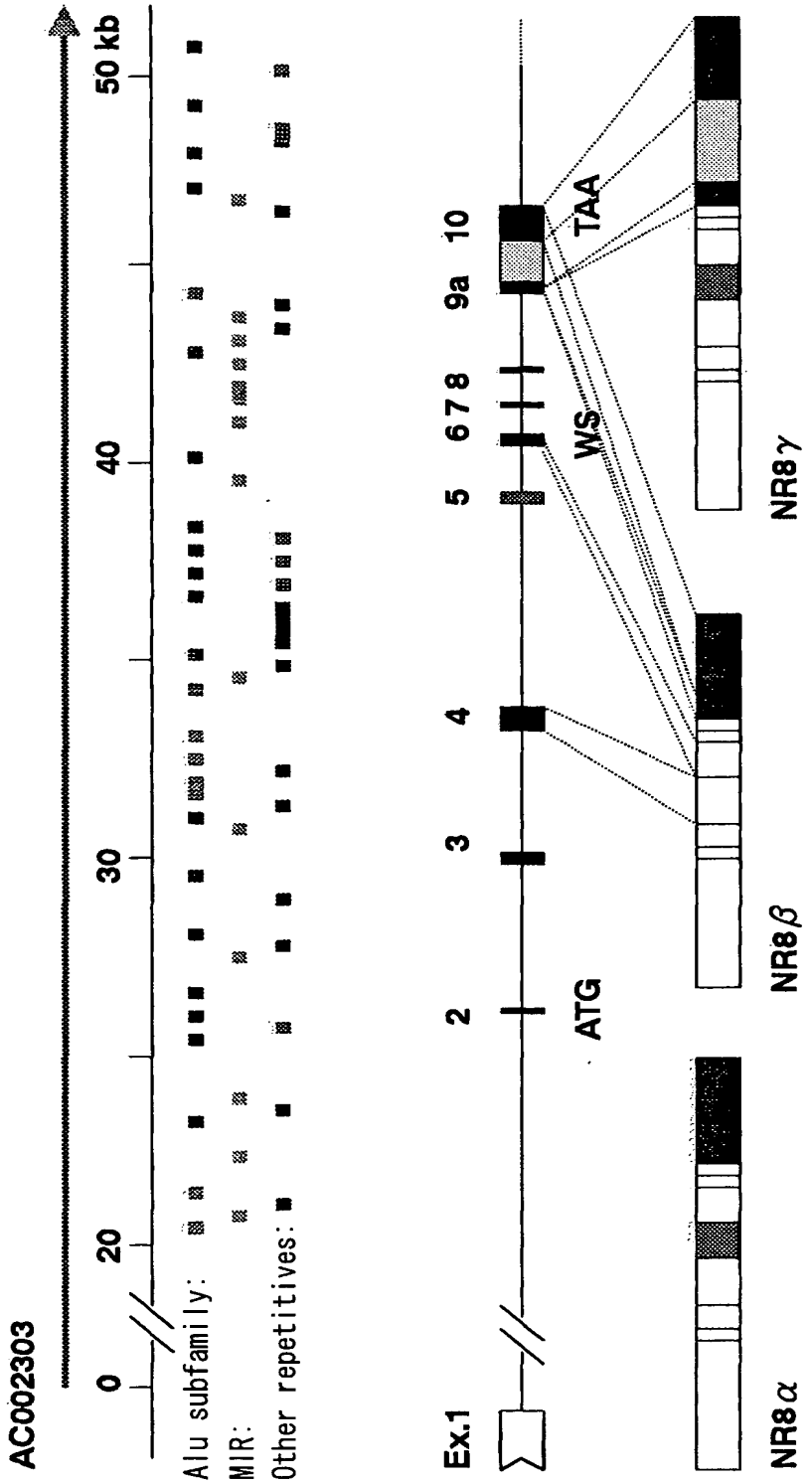
[Fig. 13]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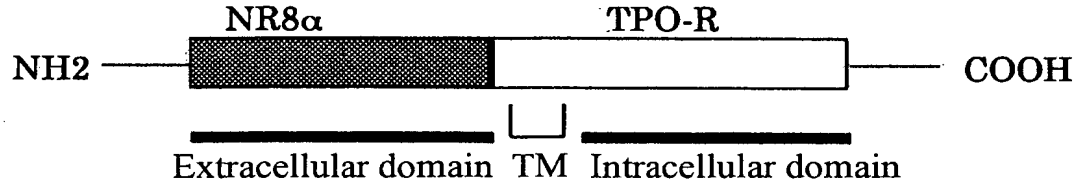
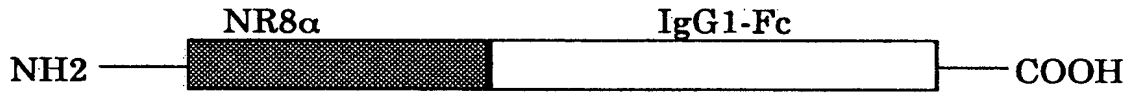
NR8β
NR8γ

[Fig. 14]

pEF-NR8/ TPO-R



pEF-NR8/ IgG-Fc



pEF-BOS/ NR8b FLAG

[Document Name] Abstract

[Abstract]

[Problems to Be Solved] The objective of the present invention is providing novel proteins, and genes encoding the proteins, and uses thereof.

[Means to Solve the Problems] The present invention provides novel hemopoietin receptor proteins (proteins comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7), proteins comprising a modified amino acid sequence of the amino acid sequence of the above protein in which one or more amino acids have been deleted, added, and/or replaced with another amino acid, genes encoding these proteins, methods of producing the proteins, as well as uses of these proteins and genes.

[Selected Drawings] None

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